EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF); Scientific Opinion on Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3): Thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3):

Thiazoles, thiophenes, thiazoline and thienyl derivatives from chemical groups 29 and 30[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 59 flavouring substances in the Flavouring Group Evaluation 21, including an additional three substances in this Revision 3, using the Procedure in Commission Regulation (EC) No 1565/2000. Since the publication of the last revision of this FGE, the EFSA has been requested to evaluate three additional substances [FL-no: 15.057, 15.079 and 15.135], which have been included in the present revision of FGE.21. Seven of the substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133] were considered to have genotoxic potential. The remaining 52 substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern, and available data on metabolism and toxicity. The Panel concluded that 26 substances [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118] do not give rise to safety concerns at their levels of dietary intake, estimated on the basis of the MSDI approach. For the remaining 26 candidate substances [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.057, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.079, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107, 15.129 and 15.135] evaluated through the Procedure, no appropriate NOAEL was available and additional data are required. Besides the safety assessment of these flavouring substances, the specifications for the

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materials of commerce have also been considered. For one substance [FL-no: 15.129], evaluated using the Procedure, an identity test is lacking and for four substances [FL-no: 15.042, 15.057, 15.079 and 15.135] the stereoisomeric composition has not been specified sufficiently.

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was asked to evaluate 59 flavouring substances in the Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000. These 59 flavouring substances belong to chemical group 29 and 30, Annex I of the Commission Regulation (EC) No 1565/2000.

The FGE.21Rev3 includes the assessment of three additional substances [FL-no: 15.057, 15.079 and 15.135] compared to the previous revision of FGE.21.

In the present FGE.21Rev3, the 59 flavouring substances include five- and six-membered sulphur-containing aromatic and non-aromatic heterocycles, which have been arranged into 11 subgroups in order to facilitate comparisons of the data sets between the groups. This division was done on the basis of degree of aromaticity and according to the presence of other heteroatoms (i.e. nitrogen).

Twelve flavouring substances possess one or more chiral centres. For four of these substances [FL-no: 15.042, 15.057, 15.079 and 15.135] the stereoisomeric composition has not been specified sufficiently.

Forty-six of the flavouring substances are classified into structural class II and 13 candidate substances are classified into structural class III, according to the decision tree approach presented by Cramer et al., 1978.

Forty-four candidate substances in the present group have been reported to occur in a wide range of food items.

In its evaluation, the Panel as a default used the "Maximised Survey-derived Daily Intake" (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a "modified Theoretical Added Maximum Daily Intake" (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its corresponding threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, the flavouring substances in this group have intakes in Europe from 0.0012 to 5.7 microgram/capita/day, which are below the thresholds of concern for structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day).

On the basis of the reported annual production volumes in Europe, the combined estimated daily per capita intakes as flavourings of the substances in subgroups with more than one substance from a structural class range from 0.14 to 8.0 microgram. For none of the subgroups do the total combined intakes exceed the thresholds of concern for compounds belonging to structural class II of 540 microgram/person/day or to structural class III of 90 microgram/person/day.

The genotoxicity data for the flavouring substances in FGE.21Rev3 are limited and genotoxicity could not be assessed adequately. However, except for two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], two thiazolidines, 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and three structurally related thiazolines, 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 52 candidate substances using the Procedure.

None of the 52 flavouring substances evaluated through the Procedure can be predicted to be metabolised to innocuous products.

Toxicological data on flavouring substances as well as data on structurally related substances were limited. Valid toxicological data which could provide an adequate margin of safety compared to the intakes from use as flavouring substances were only available for the 26 candidate substances from subgroup A-Ic (thiophenes with thiol-containing ring substituents) and subgroup A-II (thiazoles). For the remaining 26 candidate substances belonging to the subgroups of thiophene itself (A-Ia), thiophenes with non-thiol-containing ring substituents (A-Ib), benzothiazoles (A-III), dihydrothiophenes (B-I), dithiazines (B-IV) and thiadiazines (B-VI), the Panel concluded that there were insufficient data available to provide margins of safety from their use as flavouring substances and that additional toxicity data are needed.

The Panel concluded that 26 of the flavouring substances evaluated through the Procedure [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118] are not of safety concern at their estimated levels of intake based on the MSDI approach, whereas for 26 candidate substances [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.057, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.079, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107, 15.129 and 15.135], additional toxicological data are required.

The estimated intakes, based on the mTAMDI, for the 41 flavouring substances assigned to structural class II and evaluated using the Procedure, range from 78 to 4000 microgram/person/day. For one of these substances [FL-no: 15.129] the mTAMDI value of 4000 microgram/person/day is above the threshold of concern of 540 microgram/person/day for structural class II. The estimated intakes, based on the mTAMDI, for nine of the 11 candidate substances assigned to structural class III and evaluated through the Procedure, range from 78 to 250 microgram/person/day. For four of these nine substances [FL-no: 15.042, 15.055, 15.088 and 15.135] the estimated intakes are above the threshold of concern for structural class III substances of 90 microgram/person/day. For two substances [FL-no: 15.057 and 15.079] no use levels were provided. Therefore, more reliable exposure data are required for [FL-no: 15.042, 15.055, 15.057, 15.079, 15.088, 15.129 and 15.135]. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure.

In order to determine whether the conclusion for the 52 candidate substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity for the materials of commerce have been provided for 47 flavouring substances evaluated through the Procedure. An identity test has not been provided for one substance [FL-no: 15.129] and for [FL-no: 15.042, 15.057, 15.079 and 15.135] the stereoisomeric composition has not been specified sufficiently.

Thus, the final evaluation of the materials of commerce cannot be performed for five substances [FL-no: 15.042, 15.057, 15.079, 15.129 and 15.135] evaluated through the Procedure, pending further information on stereoisomerism and an identity test (for [FL-no: 15.129]).

For 26 flavouring substances evaluated through the Procedure [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.057, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.079, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107 15.129 and 15.135] the Panel considered that additional toxicity data are needed. Furthermore, for seven substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133] the Panel concluded that genotoxicity data are required.

For the remaining 26 flavouring substances [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118] evaluated using the Procedure, the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

KEYWORDS

Flavourings, safety, thiophene, thiophene derivatives, dihydrothiophene, benzothiazole derivatives thiazole derivatives, thiazoline derivatives, thiazolidine derivatives, dihydrothiazine derivatives, dithiazine derivatives, FGE.21.

TABLE OF CONTENTS

Abstract 1
Summary 2
Background 6
History of the Evaluation 6
Terms of Reference 7
Assessment 7
1. Presentation of the Substances in Flavouring Group Evaluation 21 Revision 3 7
1.1. Description 7
1.2. Stereoisomers 7
1.3. Natural Occurrence in Food 8
2. Specifications 9
3. Intake Data 9
3.1. Estimated Daily *per Capita* Intake (MSDI Approach) 10
3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI) 10
4. Absorption, Distribution, Metabolism and Elimination 12
5. Application of the Procedure for the Safety Evaluation of Flavouring Substances 16
6. Intake Estimations Based on the MSDI and the mTAMDI Approach 19
7. Considerations of Combined Intakes from Use as Flavouring Substances 20
8. Toxicity 21
8.1. Acute Toxicity 21
8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies 21
8.3. Developmental / Reproductive Toxicity Studies 25
8.4. Genotoxicity Studies 25
9. Conclusions 27
Table 1: Specification Summary of the Substances in FGE 21Rev3 30
Table 2: Summary of Safety Evaluation Applying the Procedure (Based on the MSDI Approach) 38
Table 3: Supporting Substances Summary 46
Annex I: Procedure for the Safety Evaluation 50
Annex II: Use Levels / mTAMDI 52
Annex III: Metabolism 57
Annex IV: Toxicity 76
References 84
Abbreviations 93

BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a). For the submission of data by the manufacturer, deadlines have been established by Commission Regulation (EC) No 622/2002 (EC, 2002b).

The FGE is revised to include substances for which data were submitted after the deadline as laid down in Commission Regulation (EC) No 622/2002 and to take into account additional information that has been made available since the previous Opinion on this FGE.

The Revision also includes newly notified substances belonging to the same chemical groups evaluated in this FGE.

After the completion of the evaluation programme the Union List of flavouring substances for use in or on foods in the EU shall be adopted (Article 5 (1) of Regulation (EC) No 2232/96) (EC, 1996a).

HISTORY OF THE EVALUATION

In FGE.21 and FGE.21Rev1, the Panel considered that additional toxicity data were needed for 23 of the substances (Subgroups A-Ia, A-Ib, A-III, B-I, B-IV and B-VI) evaluated through the Procedure, as no adequate toxicity study from which a no observed adverse effect level (NOAEL) could be established was available, neither on the candidate substances nor on supporting substances. Although additional toxicity data and metabolism data have become available for two substances, [FL-no: 15.106] from subgroup A-Ia and [FL-no: 15.096] from subgroup A-Ib (Flavour Industry, 2010b), in FGE.21Rev2, the Panel concluded that the data were not valid for the purposes of establishing a NOAEL.

In FGE.21Rev2, information on stereoisomeric composition provided by EFFA on nine substances [FL-no: 15.042, 15.054, 15.055, 15.060, 15.077, 15.090, 15.099, 15.119 and 15.129] (EFFA, 2010a) and followed by additional information on [FL-no: 15.054, 15.119 and 15.129] (EFFA, 2011f) was included.

Industry has informed that 11 substances [FL-no: 15.037, 15.042, 15.077, 15.088, 15.090, 15.094, 15.099, 15.107, 15.114, 15.129 and 15.133] are no longer supported for use as flavouring substances in Europe (EFFA, 2009c).

FGE	Opinion adopted by EFSA	Link	No. of candidate substances
FGE.21	8 February 2007	http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178694698331.htm	54
FGE.21Rev1	26 March 2009	http://www.efsa.europa.eu/en/scdocs/scdoc/1023.htm	56
FGE.21Rev2	4 February 2011	http://www.efsa.europa.eu/en/efsajournal/doc/1989.pdf	56
FGE.21Rev3	23 November 2011		59

The present revision of FGE.21, FGE.21Rev3, includes the assessment of three additional substances, 4,6-dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057], 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079] and ethyl thialdine [FL-no: 15.135].

No toxicity or metabolism data were provided for these substances. A search in the open literature did not reveal any further data on toxicity or metabolism for these substances.

TERMS OF REFERENCE

The European Food Safety Authority (EFSA) is requested to carry out a risk assessment on flavouring substances in the Register (Commission decision 1999/217/EC), according to Commission Regulation (EC) No 1565/2000 (EC, 2000a), prior to their authorisation and inclusion in the Union list (Regulation (EC) No 1334/2008). In addition, the Commission requested EFSA to evaluate newly notified flavouring substances, where possible, before finalising the evaluation programme. The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letter of the 12th April 2010, the Commission requested EFSA to carry out an evaluation of ethyl thialdine [FL-no: 15.135], also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a). In addition, the Commission has asked EFSA to reflect newly requested information on specifications in the revisions of FGEs.

ASSESSMENT

1. Presentation of the Substances in Flavouring Group Evaluation 21 Revision 3

1.1. Description

The present Flavouring Group Evaluation 21, Revision 3 (FGE.21Rev3), using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000 (EC, 2000a), (The Procedure –shown in schematic form in Annex I of this FGE), deals with 59 flavouring substances (candidate substances) from chemical groups 29 and 30, Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000a). The candidate substances in FGE.21Rev3 fall into the chemical groups of thiazoles (S- and N-containing), thiazolines (S- and N- containing), thienyl derivatives (S-containing), thiophenes and thiophene itself (S-containing) (see Table 4.1 in Section 4).

The candidate substances as well as their chemical Register names, FLAVIS- (FL-no), Chemical Abstract Service- (CAS-), Council of Europe- (CoE-) and Flavor and Extract Manufacturers Association- (FEMA-) numbers, structure and specifications, are listed in Table 1.

A summary of the outcome of the safety evaluation of the candidate substances are listed in Table 2.

The candidate substances are structurally closely related to 29 flavouring substances (supporting substances) evaluated at the 59th JECFA meeting (JECFA, 2002c) in the group of "Sulphur–containing heterocyclic compounds". Two of these supporting substances are mixtures (one of two isomeric isobutyl-substituted (JECFA-no: 1046) and one of two isomeric isopropyl-substituted dimethyldihydrodithiazine (JECFA-no: 1047) derivatives) and not included in the Register (see Table 3).

1.2. Stereoisomers

It is recognised that geometrical and optical isomers of substances may have different properties. Their flavour may be different; they may have different chemical properties resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be

provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers, or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Five of the candidate substances possess one chiral centre [FL-no: 15.060, 15.077, 15.090, 15.099 and 15.119], three substances possess two chiral centres [FL-no: 15.054, 15.129 and 15.135] and four possess three chiral centres [FL-no: 15.042, 15.055, 15.057 and 15.079]. For four of these substances [FL-no: 15.042, 15.057, 15.079 and 15.135] the stereoisomeric composition has not been specified sufficiently. The Flavour Industry has informed that [FL-no: 15.042] is a "mixture of diastereoisomers" (EFFA, 2010a), however, the actual ratio has to be given (see Table 1).

1.3. Natural Occurrence in Food

Forty–four candidate substances have been reported to occur naturally in one or more of the following food items: shellfish, shrimps, squid, pork, beef, lamb, chicken, vegetables, peanut, wheaten bread, butter, cheese, tea, coffee, cocoa and various types of alcoholic beverages. Quantitative data on the natural occurrence in foods have been reported for 14 of these substances (TNO, 2000; TNO, 2010; TNO, 2011), see Table 1.3.1.

1.3.1 Candidate substances reported to occur in food (TNO, 2000; TNO, 2011)

FL-no:	Name:	Quantitative data reported
15.038	2-Acetyl-4-methylthiazole	0.02 mg/kg in kohlrabi
15.040	2-Acetylthiophene	Up to 1.25 mg/kg in coffee, 0.02 mg/kg in kohlrabi, up to 0.00002 mg/kg in pork (grilled, roasted)
15.061	2,5-Dimethyl-4-ethylthiazole	0.00001 mg/kg in pork (grilled, roasted)
15.062	2,4-Dimethylthiazole	Up to 0.00004 mg/kg in pork (grilled, roasted)
15.064	2,5-Dimethylthiophene	Up to 0.01 mg/kg in papaya, up to 0.0007 mg/kg in whisky
15.069	4-Ethyl-5-methylthiazole	Up to 0.00001 mg/kg in pork (grilled, roasted)
15.076	2-Hexylthiophene	Up to 0.002 mg/kg in guinea hen
15.091	2-Methylthiophene	Up to 0.0076 mg/kg in beef (grilled, roasted), trace amounts in chicken (roasted), up to 0.1 mg/kg in papaya, 0.0009 mg/kg in shrimps (cooked), up to 0.0073 mg/kg in whisky
15.092	3-Methylthiophene	Up to 0.01 mg/kg in papaya
15.096	2-Pentylthiophene	0.001 mg/kg in chicken (roasted)
15.097	2-Propionylthiophene	0.8 mg/kg in coffee
15.106	Thiophene	0.0005 mg/kg in chicken (roasted), up to 0.8 mg/kg in tea, up to 0.0032 mg/kg in whisky
15.107	Thiophene-2-carbaldehyde	0.01 mg/kg in asparagus (cooked), 0.4 mg/kg in camembert, up to 1.8 mg/kg in coffee, up to 0.04 mg/kg in grape brandy, up to 0.03 mg/kg in malt whisky
15.135	Ethyl thialdine	0.02 mg/kg in krill, up to 0.3 mg/kg in shrimps

Fifteen of the candidate substances have not been reported to occur naturally in any food items according to TNO (TNO, 2000; TNO, 2010; TNO, 2011), see Table 1.3.2.

1.3.2 Candidate substances not reported to occur in food (TNO, 2000; TNO, 2010; TNO, 2011)

FL-no:	Name:
15.042	2-Butyl-4-methyl(4H)pyrrolidino-[1,2d]-1,3,5-dithiazine
15.044	2-Butylthiazole
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine
15.074	5-Ethylthiophene-2-carbaldehyde
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine
15.082	3-Mercaptothiophene
15.087	2-Methyl-3-mercaptothiophene
15.108	2-Thiophenemethanethiol
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine)
15.115	2-Isobutyl-4-methylthiazole
15.116	2-Acetyl-4-ethylthiazole
15.119	2-Isobutyl-3-thiazoline
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine

2. Specifications

Purity criteria for the candidate substances have been provided by the flavouring industry (EFFA, 2004i; EFFA, 2011e; Flavour Industry, 2010j). Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000a), the information is adequate for 57 substances. For two substances [FL-no: 15.129 and 15.133] an identity test is missing. Furthermore for four candidate substances [FL-no: 15.042, 15.057, 15.079 and 15.135], the stereoisomeric composition has not been specified sufficiently (see Section 1.2 and Table 1).

3. Intake Data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the "Maximised Survey-derived Daily Intake" (MSDI) by assuming that the production figure only represents 60 % of the use in food due to underreporting and that 10 % of the total EU population are consumers (SCF, 1999a).

However, the Panel noted that due to year-to-year variability in production volumes, to uncertainties in the underreporting correction factor and to uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that in contrast to the generally low *per capita* intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of products flavoured at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999a).

One of the alternatives is the "Theoretical Added Maximum Daily Intake" (TAMDI) approach, which is calculated on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as a conservative estimate of the actual intake by most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers being loyal to products flavoured at the maximum use levels reported) (EC, 2000a). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004a).

3.1. Estimated Daily *per Capita* Intake (MSDI Approach)

The intake estimation is based on the Maximised Survey-derived Daily Intake (MSDI) approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999a). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavour Industry, in which flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average *per capita* intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[4] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999a).

The total annual volume of production of the candidate substances from use as flavouring substances in Europe has been reported to be approximately 130 kg (EFFA, 2004f; EFFA, 2011e; Flavour Industry, 2010j). 4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057], 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079] and 2-acetylthiophene [FL-no: 15.040] accounts for 80 kg. For 23 of the 29 supporting substances the annual volume of production is approximately 4200 kg in Europe (JECFA, 2003a). Thiamine hydrochloride [FL-no: 16.027] accounts for 2500 kg and 5-(2-hydroxyethyl)-4-methylthiazole [FL-no: 15.014] for 1200kg. There was no reported production in Europe for five of the supporting substances in the Register [FL-no: 15.005, 15.029, 15.030, 15.109 and 15.113] and for one of the non-Register supporting substances.

On the basis of the annual volumes of production reported for the candidate substances, the daily per *capita* intakes for each of these flavourings have been estimated (Table 2).

The estimated daily *per capita* intake of 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079], from use as a flavouring substance is 5.7 microgram, of 2-acetylthiophene [FL-no: 15.040] 2.2 microgram, of 4,6-dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057] 1.5 microgram and of 4-butylthiazole [FL-no: 15.118] 1.3 microgram. For the remaining 55 substances, the estimated daily *per capita* intakes are in the range of 0.0012 to 0.61 microgram (see Table 2).

3.2. Intake Estimated on the Basis of the Modified TAMDI (mTAMDI)

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

[4] EU figure 375 millions. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

For the present evaluation of the candidate substances, information on food categories and normal and maximum use levels[5,6,7] were submitted by the Flavour Industry (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5; Flavour Industry, 2010j) for 57 substances. No information on use levels have been submitted for [FL-no: 15.057 and 15.079]. The candidate substances are used in flavoured food products divided into the food categories outlined in Annex III of the Commission Regulation (EC) No 1565/2000 (EC, 2000a), as shown in Table 3.1. For the present calculation of the mTAMDI, the reported normal use levels were used. In the case where different use levels were reported for different food categories the highest reported normal use level was used.

Table 3.1 Use of Candidate Substances in various food categories for 57 candidate substances for which data on use have been provided.

Food category	Description	Flavourings used*
01.0	Dairy products, excluding products of category 2	All
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All
03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 15.094 and 15.135]
04.1	Processed fruits	All
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	[FL-no: 15.062, 15.129,15.133 and 15.135]
05.0	Confectionery	All except [FL-no: 15.062 and 15.135]
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	All
07.0	Bakery wares	All
08.0	Meat and meat products, including poultry and game	All
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-no: 15.039 and 15.135]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	None
12.0	Salts, spices, soups, sauces, salads, protein products etc.	All except [FL-no: 15.089]
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-no: 15.129, 15.133 and 15.135]
14.1	Non-alcoholic ("soft") beverages, excl. dairy products	All except [FL-no: 15.135]
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts	All except [FL-no: 15.062 and 15.135]
15.0	Ready-to-eat savouries	All except [FL-no: 15.068]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 1 – 15	All except [FL-no: 15.135]

* No use levels have been submitted for [FL-no: 15.057 and 15.079]

According to the Flavour Industry the normal use levels are in the range of 0.1-10 mg/kg food, and the maximum use levels are in the range of 0.2-250 mg/kg (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5; Flavour Industry, 2010j).

The mTAMDI values for the 46 candidate substances from structural class II (see Section 5) range from 78 to 4000 microgram/person/day. For the remaining 11 candidate substances from structural class III for which data have been provided, the mTAMDI range from 78 to 4000 microgram/person/day.

[5] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i).

[6] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004e).

[7] The use levels from food category 5 "Confectionery" have been inserted as default values for food category 14.2 "Alcoholic beverages" for substances for which no data have been given for food category 14.2 (EFFA, 2007a).

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 6 and Annex II.

4. Absorption, Distribution, Metabolism and Elimination

The 59 candidate substances are structurally related to 29 supporting substances evaluated by the JECFA in "Sulfur-containing heterocyclic compounds" (JECFA, 2003a). The substances are divided into 11 subgroups based on the nature of the ring (aromatic (clustered in subgroups A-Ia,-b,-c, A-II and A-III) *vs.* non-aromatic (clustered in subgroups B-I to B-VI)) depending on type and number of ring heteroatoms (sulphur or sulphur with nitrogen), and the degree of saturation in the non-aromatic rings. The assignment of the individual substances to the different subgroups is presented in Table 4.1. The structures of the substances and a description of the characteristic features of the 11 subgroups are also given in Table III.1 and the accompanying text in Annex III.

TABLE 4.1 DIVISION OF CANDIDATE SUBSTANCES INTO STRUCTURAL SUBGROUPS

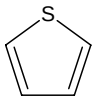
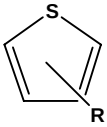
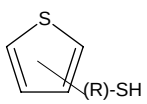
Subgroup and common ring structure	Register name	FL-no
A Aromatic subgroups		
A-Ia: Thiophene 	Thiophene	15.106
A-Ib: Thiophenes: (with non-thiol-containing ring substituents) 	2-Methylthiophene	15.091
	3-Methylthiophene	15.092
	2-Ethylthiophene	15.072
	2-Butylthiophene	15.045
	2-Pentylthiophene (Register name: *sec*-Pentylthiophene)	15.096
	2-Hexylthiophene	15.076
	2-Octylthiophene	15.093
	2,5-Dimethylthiophene	15.064
	2-Ethyl-5-methylthiophene	15.070
	2-Butyl-5-ethylthiophene	15.043
	2-Acetylthiophene	15.040
	2-Propionylthiophene	15.097
	2-Pentanoylthiophene	15.094
	2-Acetyl-3-methylthiophene	15.037
	Thiophene-2-carbaldehyde	15.107
	5-Ethylthiophene-2-carbaldehyde	15.074
A-Ic: Thiophenes: (with thiol-containing ring substituents) 	3-Mercaptothiophene	15.082
	2-Thiophenemethanethiol	15.108
	2-Methyl-3-mercaptothiophene	15.087
A-II: Thiazoles	2-Methylthiazole	15.089
	2-Ethylthiazole	15.071
	2-Propylthiazole	15.098
	2-Butylthiazole	15.044
	4-Butylthiazole	15.118

TABLE 4.1 DIVISION OF CANDIDATE SUBSTANCES INTO STRUCTURAL SUBGROUPS

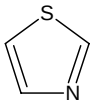
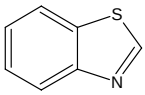
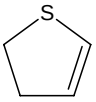
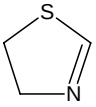
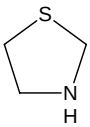
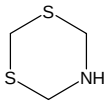
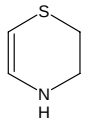
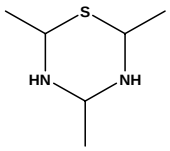
Subgroup and common ring structure	Register name	FL-no
	2,4-Dimethylthiazole	15.062
	2,5-Dimethylthiazole	15.063
	5-Ethyl-2-methylthiazole	15.068
	4-Ethyl-2-methylthiazole	15.067
	4-Ethyl-5-methylthiazole	15.069
	2,5-Diethylthiazole	15.052
	5-Methyl-2-pentylthiazole	15.084
	4,5-Dimethyl-2-ethylthiazole	15.058
	2,5-Dimethyl-4-ethylthiazole	15.061
	2,5-Diethyl-4-methylthiazole	15.050
	2,5-Diethyl-4-propylthiazole	15.051
	2-Isobutyl-4-methylthiazole	15.115
	2-Isobutyl-4,5-dimethylthiazole	15.078
	2-Isopropyl-4,5-dimethylthiazole	15.080
	2-Acetyl-4-methylthiazole	15.038
	2-Acetyl-5-methylthiazole	15.039
	2-Acetyl-4-ethylthiazole	15.116
	4-Methyl-2-propionylthiazole	15.085
A-III: Benzothiazoles	2-Methyl-4,5-benzothiazole	15.088
		
B Non-aromatic subgroups:		
B-I : Dihydrothiophenes	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	15.077
		
B-II: Thiazolines	2-Methyl-2-thiazoline	15.086
	2,4-Dimethyl-3-thiazoline	15.060
	2-Isobutyl-3-thiazoline	15.119
		
B-III: Thiazolidines	2-Methylthiazolidine	15.090
	2-Propylthiazolidine	15.099
		
B-IV: Dithiazines	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	15.054
	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	15.055
	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	15.042
	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	15.057
	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	15.079

TABLE 4.1 DIVISION OF CANDIDATE SUBSTANCES INTO STRUCTURAL SUBGROUPS

Subgroup and common ring structure	Register name	FL-no
	Ethyl thialdine	15.135
B-V: Dihydrothiazines	6-Acetyl-2,3-dihydro-1,4-thiazine (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine)	15.114
	5-Acetyl-2,3-dihydro-1,4-thiazine	15.133
B-VI: Thiadiazine	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	15.129
		

From the few data available on absorption, distribution and elimination of the aromatic candidate substances in this FGE, it is anticipated that they may be absorbed and eliminated after biotransformation. Some volatile substances may also be eliminated unchanged via exhalation. For the non-aromatic substances there are no data available for the candidate or for the supporting substances.

For the evaluation of the metabolism of the candidate substances, only limited data were available. These were confined to a few studies on thiophene, and some thiophene-, thiazole- and benzothiazole-derivatives (i.e. only directly relevant for the evaluation of subgroups-A (A-Ia, -Ib, -Ic, -II, -III)), the aromatic candidate substances. Other information was found in several review papers. However, virtually no data were found on the metabolism of substances from the B-subgroups (B-I, -II, -III, -IV, -V, -VI) possessing non-aromatic ring structures.

Metabolism of substances in subgroup A-I

Thiophene and the ring-substituted thiophenes (A-Ia, b and c) may undergo S-oxidation to give sulphoxides. These primary metabolites may react spontaneously or via glutathione transferase with glutathione (GSH), and it is likely that they also exhibit reactivity towards protein thiols. The glutathione conjugates may be further metabolised to the corresponding mercapturic acids and excreted in the urine. S-oxide intermediates may also be subject to dimerisation via a Diels-Alder reaction. In addition, 4,5-epoxide formation has been reported for 2-phenylthiophene, followed by subsequent conjugation of the epoxide with glutathione. This may also be anticipated for other ring-substituted thiophenes.

Only very limited information was submitted to show whether side chain oxidation of the ring-substituted thiophenes could also occur. Based on some studies with a substituted thiazole (chlormethiazole) and one (heavily) substituted thiophene (olanzapine), such side chain reactions may be anticipated. For the substances in subgroup A-Ib, metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. with glucuronic acid. Similar reactions (e.g. omega or omega-1 oxidations) for ring-substituent chains have been discussed for alkylated pyrazines (EFSA, 2010h). The two thiophene carbaldehyde candidate substances [FL-no: 15.107 and 15.074] may be expected to

be oxidised to the corresponding carboxylic acids. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (see FGE.13Rev2 (EFSA, 2011h); FGE.14Rev1 (EFSA, 2009f); FGE.17Rev2 (EFSA, 2010h)). The acyl-substituted thiophenes [FL-no: 15.037, 15.040, 15.094 and 15.097] may be subject to keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furans (EFSA, 2011h) and of pyrazines (EFSA, 2010h).

The mercapto-group in the ring-substituent of the substances in subgroup A-Ic may undergo S-methylation to produce the corresponding methyl thioether, with further oxidation to the corresponding sulphoxide and sulphone. They may also react with glutathione or other endogenous thiol substances to form mixed disulphides, which may undergo reduction to thiols or oxidative desulphuration. Alternatively, they may undergo enzymatic oxygenation resulting in the formation of the corresponding sulphinic or sulphonic acids. These metabolites are all expected to be excreted in the urine. A more detailed discussion on the metabolism of sulphur compounds can be found in FGE.13Rev2 (EFSA, 2011h).

Metabolism of substances in subgroup A-II

Thiazoles may be subject to ring S- and N-oxidation. From the various studies with thiazole derivatives (A-II), it can be seen that their metabolites may react spontaneously with glutathione, and it is likely that they also exhibit reactivity towards biomacromolecules. In addition, for some thiazole derivatives, ring C-oxidation may be accompanied by heterocyclic ring cleavage which can result in the formation of alpha-diketone and thioamide intermediates. For the latter a relationship with nephro- and hepatotoxicity has been established, especially after GSH depletion, but this is a high-dose phenomenon. Due to the compound specificity of these reactions, the extent of formation cannot be generalised, however, these thioamide intermediates and the S-oxides seem to be quantitatively minor metabolites.

Similarly to the situation with the ring-substituted thiophenes, only very limited information was available which could demonstrate whether side chain oxidation of the ring-substituted thiazoles could occur. Based on some studies with a substituted thiazole (chlormethiazole) such side chain reactions may be anticipated. Metabolites of the side chain oxidation pathways may be expected to be conjugated, e.g. with glucuronic acid. Similar reactions (e.g. omega or omega-1 oxidations) for ring-substituent chains have been discussed for alkylated pyrazines (EFSA, 2010h). The acyl-substituted thiazoles [FL-no: 15.038, 15.039, 15.085 and 15.116] may be subject to keto-reduction, possibly followed by conjugation, similarly to acetyl derivatives of furanes (EFSA, 2011h) and pyrazines (EFSA, 2010h).

Metabolism of substances in subgroup A-III

In addition to ring cleavage as mentioned for the thiazoles in subgroup A-II, oxidation of the fused benzene ring has also been reported for some structural analogues of the candidate substance in subgroup A-III, 2-methyl-4,5-benzothiazole, [FL-no: 15.088]. No further information was available on the metabolism of this substance or on structurally related substances.

Conclusions for the substances in the subgroups A-I to A-III

From the very limited data available on the aromatic subgroups (A) it is not anticipated that the thiophenes (A-I) and thiazoles (A-II) utilise similar metabolic pathways. These sulphur-containing heteroaromatic derivatives can be expected to participate in metabolic pathways principally involving side-chain C-oxidation, epoxidation of double bonds, oxidation of the ring-S and (for the thiazoles) ring-N to yield sulphoxide or sulphones and N-oxides, respectively. The reactive intermediates (e.g. epoxides and S-oxides) may be conjugated with glutathione; the glutathione conjugates may be excreted directly in the urine or following further biotransformation to the corresponding mercapturic acids. Furthermore, from the data available it is anticipated that the unsubstituted thiophene (A-Ia) and benzothiazole (A-III) are metabolised differently from their ring-substituted derivatives.

In the light of the above considerations and as a consequence of the expected reactivity of the thiol groups and the possible reactions of the ring side-chains it cannot be anticipated that the substances in the aromatic subgroups (A) are metabolised to innocuous substances. The Panel was aware of the potential bio-activation of candidate substances with heteroaromatic rings to yield intermediates via ring-scission or S/N-oxidation which could be reactive to proteins or DNA. However, taking into account available data on structurally related thiazoles and considering the available genotoxicity data on thiophenes and thiazoles in the present revision of FGE.21 (FGE.21Rev3) (negative Ames tests and a chromosome aberration test), the Panel concluded that there are not sufficient indications to preclude the application of the Procedure to the thiophenes, thiazoles and benzothiazole (A-I to A-III).

Metabolism of the substances in subgroups B-I to B-VI

No specific information was available on the metabolism of the dihydrothiophene-, thiazoline-, thiazolidine-, dithiazine-, dihydrothiazine-, thiadiazine-derivatives or related substances for any of these non-aromatic substances in the B-subgroups. It may be speculated that the substances in these groups are metabolised primarily by oxidation of the ring-S, or, if applicable, *via* N-oxidation. In addition, metabolism of the ring substituents is likely to occur.

The substances exhibiting thioacetal structures could be subject to acid hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant than oxygen acetals. It is thus to be anticipated that these substances may reach the intestinal lumen intact and may also be absorbed as such.

Conclusions for the substances in the subgroups B-I to B-VI

Due to the lack of metabolism data it cannot be concluded that the candidate substances in the B-subgroups will be metabolised to innocuous products.

5. Application of the Procedure for the Safety Evaluation of Flavouring Substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 6.

For the two candidate substance 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] from subgroup B-V, which are alpha,beta-unsaturated ketones, i.e. they have a structural alert for genotoxicity (EFSA, 2008b), there are no genotoxicity data available and accordingly a concern for genotoxicity could not be ruled out. For the two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] from subgroup B-III, there are indications of a genotoxic potentials *in vitro*. Considering the structural similarities between these two thiazolidines in subgroup B-III and the three thiazolines in subgroup B-II (2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119]), the Panel concluded that in the absence of further genotoxicity data the Procedure could not be applied to these five substances from subgroup B-II and B-III.

Thus, the Panel concluded that in the absence of further genotoxicity data the Procedure could not be applied to the seven substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133].

For the safety evaluation of the remaining 52 candidate substances from chemical groups 29 and 30 the Procedure as outlined in Annex I was applied, based on the MSDI approach. The outcome of the evaluations of the 52 substances are summarised in Table 2.

Step 1

According to the decision tree approach, presented by Cramer et al. (Cramer et al., 1978) 41 of the candidate substances, evaluated through the Procedure, are classified into structural class II and 11 into structural class III, see Table 2.

Step 2

None of the 52 candidate substances can be predicted to be metabolised to innocuous products. Therefore, the evaluation of all 52 candidate substances proceeds *via* the B-side of the Procedure.

Step B3

The 41 candidate substances in structural class II have estimated European daily *per capita* intakes (MSDI) ranging from 0.0012 microgram to 2.2 microgram (Tables 2 and 6.1). These intakes are below the threshold of concern of 540 microgram/person/day for structural class II substances.

Similarly, the estimated daily *per capita* intakes of all 11 candidate substances in structural class III, ranging from 0.0012 microgram to 5.7 microgram (Tables 2 and 6.1), are below the threshold of concern for structural class III substances of 90 microgram/person/day.

Accordingly, the evaluation of all 52 candidate substances proceeds to step B4.

Step B4

Subgroup A-Ia: Thiophene

In the initial assessments of this flavouring group (FGE.21 (EFSA, 2008s), FGE.21Rev1 (EFSA, 2009u) and FGE.21Rev2 (EFSA, 2011af), no valid toxicity study from which a No Observed Adverse Effect Level (NOAEL) could be established was available for the candidate substance or for any relevant supporting substance. Accordingly, further data were required for thiophene [FL-no: 15.106]. Although additional toxicity data were provided by Industry on thiophene (Flavour Industry, 2010b), the Panel considered that these data were not valid for the purposes of establishing a NOAEL to be used in the Procedure. Accordingly further data are still required for thiophene.

Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents

In the initial assessments of this flavouring group (FGE 21 (EFSA, 2008s) and FGE 21Rev1 (EFSA, 2009u) and FGE.21Rev2 (EFSA, 2011af), no valid toxicity study from which a NOAEL could be established was available for the candidate substances or for any relevant supporting substances. Therefore, the Panel concluded that additional toxicity data are needed for the 16 substituted thiophenes in subgroup A-Ib. Although additional toxicity data were provided by Industry on the candidate substance 2-pentylthiophene (Register name: *sec*-pentylthiophene) [FL-no: 15.096] (Flavour Industry, 2010b), the Panel considered that these data were not valid for the purposes of establishing a relevant NOAEL for the subgroup. Accordingly additional toxicity data are still needed for the 16 substituted thiophenes [FL-no: 15.037, 15.040, 15.043, 15.045, 15.064, 15.070, 15.072, 15.074, 15.076, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097 and 15.107] in subgroup A-Ib.

Subgroup A-Ic: Thiophenes with thiol-containing ring substituents

A NOAEL of 0.29 mg/kg body weight (bw)/day was reported for the supporting substance 2-thienyl disulfide [FL-no: 15.008] in a single-dose level 90-day study in rats. The combined estimated daily *per capita* intake of 0.14 microgram for the three candidate substances in subgroup A-Ic corresponds to 0.0023 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 1.3×10^5 can be calculated**.**

On the basis of the application of the Procedure, 3-mercaptothiophene [FL-no: 15.082], 2-methyl-3-mercaptothiophene [FL-no: 15.087] and 2-thiophenemethanethiol [FL-no: 15.108] are not expected to be of safety concern at their estimated levels of intake.

Subgroup A-II: Thiazoles

A NOAEL of 25 mg/kg bw/day was reported for the supporting substance 5-acetyl-2,4-dimethylthiazole [FL-no: 15.011] in a single-dose level 90-day study in rats. The combined estimated daily *per capita* intake of 2.3 microgram for the 23 candidate substances in subgroup A-II corresponds to 0.038 microgram/kg bw/day at a body weight of 60 kg. Thus, a margin of safety of 6.6×10^5 can be calculated**.**

On the basis of the application of the Procedure, the 23 candidate substances in subgroup A-II are not expected to be of safety concern at their estimated levels of intake.

Subgroup A-III: Benzothiazoles

There were no valid toxicological data available on the candidate substance 2-methyl-4,5-benzothiazole [FL-no: 15.088] nor for any sufficiently structurally related substances. The Panel considered that data on the supporting substance benzothiazole [FL-no: 15.016] could not be used in the Procedure for 2-methyl-4,5-benzothiazole, since the unsubstituted benzothiazole is anticipated to be metabolised differently from the substituted benzothiazole in subgroup A-III. Therefore, the Panel concluded that additional toxicity data are needed for 2-methyl-4,5-benzothiazole [FL-no: 15.088].

Subgroup B-I: Dihydrothiophenes

No toxicological data were available on 4-hydroxy-2,5-dimethylthiophen-3(2H)-one [FL-no: 15.077] or for structurally related supporting substances. Therefore, the Panel concluded that additional toxicity data are needed for this substance.

Subgroup B-II: Thiazolines

The candidate substances were not evaluated through the Procedure.

Subgroup B-III: Thiazolidines

The candidate substances were not evaluated through the Procedure.

Subgroup B-IV: Dithiazines

There were no toxicological data available on the candidate substances in this subgroup and the Panel concluded that the data available on supporting substances could not be used for deriving a NOAEL to support the candidate substances in this subgroup B-IV. Accordingly, additional data are needed for 2-butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine [FL-no: 15.042], dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine [FL-no: 15.054], 2,4-dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine [FL-no: 15.055], 4,6-dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine [FL-no: 15.057], 2-isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine [FL-no: 15.079] and ethyl thialdine [FL-no: 15.135] in subgroup B-IV.

Subgroup B-V: Dihydrothiazines

The candidate substances were not evaluated through the Procedure.

Subgroup B-VI: Thiadiazines

No toxicological data were available on tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129], or on structurally related supporting substances. Therefore, the Panel concluded that additional toxicity data are needed for this substance from subgroup B-VI.

Summary of application of the Procedure

Of the 52 substances evaluated through the Procedure, in total, 26 of the candidate substances are not of safety concern at their estimated levels of intake based on the MSDI approach whereas for 26 candidate substances additional data are required (see Table 2).

6. Intake Estimations Based on the MSDI and the mTAMDI Approach

The estimated intakes, based on the mTAMDI, for the 41 candidate substances assigned to structural class II and evaluated using the Procedure range from 78 to 4000 microgram/person/day. For one of the 41 substances, tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129], the mTAMDI value of 4000 microgram/person/day is above the threshold of concern of 540 microgram/person/day for a structural class II substance.

The estimated intakes, based on the mTAMDI, for nine candidate substances assigned to structural class III and evaluated using the Procedure, range from 78 to 250 microgram/person/day. For four of these candidate substances (2-butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine [FL-no: 15.042], 2,4-dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine [FL-no: 15.055], 2-methyl-4,5-benzothiazole [FL-no: 15.088] and ethyl thialdine [FL-no: 15.135] the mTAMDI values are above the threshold of concern for structural class III substances of 90 microgram/person/day. For two candidate substances [FL-no: 15.057 and 15.079] assigned to structural class III, no information on use levels have been provided. For comparison of the MSDI and mTAMDI values, see Table 6.1.

Further information is therefore required for the seven substances [FL-no: 15.042, 15.055, 15.057, 15.079, 15.088, 15.129 and 15.135]. This would include more reliable intake data. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this procedure additional toxicological data might become necessary.

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
15.037	2-Acetyl-3-methylthiophene	0.18	78	Class II	540
15.038	2-Acetyl-4-methylthiazole	0.0049	160	Class II	540
15.039	2-Acetyl-5-methylthiazole	0.0024	160	Class II	540
15.040	2-Acetylthiophene	2.2	78	Class II	540
15.043	2-Butyl-5-ethylthiophene	0.0012	78	Class II	540
15.044	2-Butylthiazole	0.011	160	Class II	540
15.045	2-Butylthiophene	0.012	78	Class II	540
15.050	2,5-Diethyl-4-methylthiazole	0.012	160	Class II	540
15.051	2,5-Diethyl-4-propylthiazole	0.0012	160	Class II	540
15.052	2,5-Diethylthiazole	0.015	160	Class II	540
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	0.0012	160	Class II	540
15.058	4,5-Dimethyl-2-ethylthiazole	0.015	130	Class II	540
15.061	2,5-Dimethyl-4-ethylthiazole	0.011	160	Class II	540
15.062	2,4-Dimethylthiazole	0.61	140	Class II	540
15.063	2,5-Dimethylthiazole	0.0061	160	Class II	540
15.064	2,5-Dimethylthiophene	0.23	78	Class II	540
15.067	4-Ethyl-2-methylthiazole	0.0037	160	Class II	540
15.068	5-Ethyl-2-methylthiazole	0.0061	220	Class II	540
15.069	4-Ethyl-5-methylthiazole	0.012	160	Class II	540
15.070	2-Ethyl-5-methylthiophene	0.061	78	Class II	540
15.071	2-Ethylthiazole	0.028	160	Class II	540
15.072	2-Ethylthiophene	0.0012	78	Class II	540
15.074	5-Ethylthiophene-2-carbaldehyde	0.0012	78	Class II	540
15.076	2-Hexylthiophene	0.12	78	Class II	540
15.078	2-Isobutyl-4,5-dimethylthiazole	0.12	160	Class II	540
15.080	2-Isopropyl-4,5-dimethylthiazole	0.012	160	Class II	540
15.084	5-Methyl-2-pentylthiazole	0.0037	160	Class II	540
15.085	4-Methyl-2-propionylthiazole	0.0037	160	Class II	540
15.089	2-Methylthiazole	0.018	150	Class II	540
15.091	2-Methylthiophene	0.019	78	Class II	540
15.092	3-Methylthiophene	0.12	78	Class II	540
15.093	2-Octylthiophene	0.012	160	Class II	540
15.094	2-Pentanoylthiophene	0.0012	160	Class II	540

Table 6.1 Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/*capita*/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
15.096	sec-Pentylthiophene	0.24	160	Class II	540
15.097	2-Propionylthiophene	0.12	160	Class II	540
15.098	2-Propylthiazole	0.085	160	Class II	540
15.107	Thiophene-2-carbaldehyde	0.21	78	Class II	540
15.115	2-Isobutyl-4-methyl thiazole	0.011	160	Class II	540
15.116	2-Acetyl-4-ethylthiazole	0.024	160	Class II	540
15.118	4-Butylthiazole	1.3	160	Class II	540
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	0.61	4000	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	0.012	160	Class II	540
15.086	2-Methyl-2-thiazoline	0.24	160	Class II	540
15.090	2-Methylthiazolidine	0.024	160	Class II	540
15.099	2-Propylthiazolidine	0.012	160	Class II	540
15.119	2-Isobutyl-3-thiazoline	0.011	160	Class II	540
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	0.0012	160	Class III	90
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	0.055	160	Class III	90
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	1.5		Class III	90
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	0.12	78	Class III	90
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	5.7		Class III	90
15.082	3-Mercaptothiophene	0.011	78	Class III	90
15.087	2-Methyl-3-mercaptothiophene	0.12	78	Class III	90
15.088	2-Methyl-4,5-benzothiazole	0.0085	160	Class III	90
15.106	Thiophene	0.12	78	Class III	90
15.108	2-Thiophenemethanethiol	0.0073	78	Class III	90
15.135	Ethyl thialdine	0.61	250	Class III	90
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine	0.012	160	Class III	90
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	0.61	4000	Class III	90

7. Considerations of Combined Intakes from Use as Flavouring Substances

Because of structural similarities of the candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in case of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are only based on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily *per capita* intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

Seven of the candidate substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133] have not been evaluated through the Procedure (see Section 5) and are therefore not considered together with the other 52 candidate substances evaluated in FGE.21Rev3 in the combined intake.

On the basis of the reported annual production volumes in Europe (EFFA, 2004f; EFFA, 2011e; Flavour Industry, 2010j), the combined estimated daily *per capita* intakes as flavourings of the candidate substances in subgroups, with more than one substance from a structural class (SC), are: subgroup A-Ib (16 substances from SC II) 3.6 microgram; subgroup A-Ic (three substances from SC III) 0.14 microgram; subgroup A-II (23 substances from SC II) 2.3 microgram; and subgroup B-IV (five substances from SC III) 8.0 microgram.

The 52 candidate substances evaluated through the Procedure, are structurally related to 29 supporting substances evaluated by the JEFCA at its 59th JECFA meeting (JECFA, 2002c). The total combined daily *per capita* intakes (in Europe) of candidate and supporting substances in each of the four subgroups for which supporting substances were available are: subgroup A-Ib (18 substances from SC II) 22 microgram; subgroup A-Ic (five substances from SC III) 0.15 microgram; subgroup A-II (36

substances from SC II) 500 microgram and subgroup A-III (two substances from SC III) 1.2 microgram.

For none of the subgroups do the total combined intakes exceed the thresholds of concern for compounds belonging to structural class II of 540 microgram/person/day or to structural class III of 90 microgram/person/day.

8. Toxicity

8.1. Acute Toxicity

Data are available for two candidate substances, thiophene [FL-no: 15.106] and thiophene-2-carbaldehyde [FL-no: 15.107] and for 17 of the 29 supporting substances. The Oral LD_{50} values in rats or mice ranged from 180 to 3700 mg/kg body weight (bw).

The acute toxicity data are summarised in Annex IV, Table IV.1

8.2. Subacute, Subchronic, Chronic and Carcinogenicity Studies

Subacute toxicity data were available for three of the candidate substances, thiophene [FL-no: 15.106], 2-pentylthiophene (Register name: *sec*-pentylthiophene) [FL-no: 15.096] and thiophene-2-carbaldehyde [FL-no: 15.107] and for 11 supporting substances, of which nine is in the Register [FL-no: 15.002, 15.004, 15.005, 15.008, 15.011, 15.016, 15.020, 15.024 and 15.029] and two are mixtures of structurally related substances (one mixture of 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine, and one mixture of 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine).

The repeated dose toxicity studies are summarised in Annex IV, Table IV.2

Subgroup A-Ia: Thiophene

Based on the available data on metabolism, the toxicity of the candidate substance thiophene [FL-no: 15.106] is considered separately from the substituted thiophenes in subgroup A-Ib. Two short-term toxicity tests are available on thiophene. In a study, in which thiophene was administered to groups of 5 male rats at dose levels of 0 - 1000 mg/kg bw/day by gavage for up to 19 days, evidence of central nervous system toxicity was seen in higher dose groups, accompanied by haematological and clinical chemistry changes and increased relative liver weights (O'Donoghue, 1979). Histologically, hepatic necrosis was present in the 500 and 1000 mg/kg bw groups and one rat in the 1000 mg/kg bw groups showed thymic cortical atrophy, splenic lymphoid depletion and hypocellular bone marrow. The reported No Observed Adverse Effect Level (NOAEL) was 100 mg/kg bw/day. The Panel considered that this study was inadequate for deriving an appropriate NOAEL to be used in the Procedure because of the short duration and the limited study details given.

A combined repeat dose/reproductive and developmental toxicity study, in accordance with OECD Test Guideline 422, has been carried out with thiophene, in which males were dosed for 42 days and females from 14 days before mating until Day 3 of lactation (Nagao, 2006). The full report of this study was in Japanese, however, a summary of the study design, results and conclusions to be drawn were available to the Panel in English. Groups of Sprague-Dawley rats (13/sex/dose) were administered thiophene *via* gavage at doses of 0, 25, 100 and 400 mg/kg/day. Rats of both sex showed ataxia after receiving 100 mg/kg of thiophene or more. Decreased food consumption and decreased body weight gain was observed in the 400 mg/kg group of both sexes when compared to the controls. Clinical chemistry revealed decreases in glucose levels and alkaline phosphatase activity and an increase in inorganic phosphorous levels for males in the 100 and 400 mg/kg bw/day groups. At necropsy, increases in relative liver weights compared with controls were statistically significant in

males in the 100 and 400 mg/kg bw/day groups and in females receiving 400 mg/kg bw/day. Histopathological evidence of liver damage was noted for males and females given 100 or 400 mg/kg bw/day. In both males and females receiving 400 mg/kg bw/day these included hepatocellular hypertrophy, inflammatory change, hepatocellular necrosis and a homogenous or vesicular cytoplasmic change of the hepatocytes in the central zones, while at 100 mg/kg bw/day, changes consisted of hepatocellular hypertrophy and some inflammatory change. A high incidence of histopathological changes were reported in the cerebellum for female rat given 400 mg/kg bw/day, including pyknosis or necrosis of the granular cells, necrosis of the *laminae albae* of the brain and degeneration or loss of Purkinje cells. Pyknosis or necrosis of the granular cells was also observed in one female receiving 100 mg/kg bw/day and one male receiving 400 mg/kg bw/day. In females, relative kidney weights were increased when compared to controls in the 100 and 400 mg/kg bw/day groups and a vacuolar degeneration of the tubular epithelium was observed. Based on these findings the authors of this study reported a NOAEL of 25 mg/kg bw/day (Nagao, 2006). The Panel considered that this study was inadequate for deriving an appropriate NOAEL to be used in the Procedure because of the short duration. According to the practice of the Panel, a minimum requirement to provide an adequate NOAEL for flavourings in the Procedure is a 90-day study.

Subgroup A-Ib: Thiophenes with non-thiol-containing ring substituents

In a study conducted in accordance with OECD Test Guideline 407, 2-Pentylthiopene (Register name: *sec*-pentylthiophene) [FL-no: 15.096] was administered by gavage to three groups each of five male and five female Sprague-Dawley Crl:CD® (SD) IGS BR strain rats, for twenty-eight days, at dose levels of 15, 150 or 500 mg/kg bw/day (Dhinsa et al., 2006). A control group of five males and five females was dosed with vehicle alone (Arachis oil). Clinical signs during treatment included increased salivation in all animals treated with 500 mg/kg bw/day and the majority of animals treated with 150 mg/kg bw/day. A slight reduction in body weight gain was observed in males only treated with 500 mg/kg bw/day, while males and females treated with 500 mg/kg bw/day showed an increase in water consumption. Haematologic examination revealed haemolytic anaemia in animals treated with 500 and 150 mg/kg bw/day as evidenced by reductions in haemoglobin, haematocrit and erythrocyte count and evidence of reticulocytosis. Clinical chemical determinations on day 28 showed increases in bilirubin and alanine aminotransferase for animals of both sexes treated with 500 mg/kg bw/day, with increases in bilirubin also noted for both sexes animals treated with 150 mg/kg/day, and in females receiving 15 mg/kg bw/day. Absolute and relative liver, kidney and spleen weights were increased in both sexes treated with 500 mg/kg bw or 150 mg/kg bw/day per day. At necropsy animals in these dose groups had dark and enlarged spleens. Histopathological examination revealed hepatocellular hypertrophy in animals of both sexes treated with 500 and 150 mg/kg bw/day. Histopathological evidence of extramedullary haemopoiesis and haemosiderin accumulation in the spleen of animals of either sex treated with 500 and 150 mg/kg bw/day, together with observation of haemosiderin deposits in the liver and kidney, were typical of a haemolytic anaemia, as also evidenced by the haematological measurements. There was also some evidence of renal damage in males and females treated with 500 and 150 mg/kg bw/day, as evidenced in occasional animals only by tubular hypertrophy, tubular dilatation and tubular basophilia, pyelitis with associated hyperplasia of the renal papillary/pelvic epithelium (females only) and globular accumulations of eosinophilic material in the renal tubules (males only). Epithelial hyperplasia of the urinary bladder and associated epithelial and subepithelial inflammatory cell infiltrates were observed for two females treated with 500 mg/kg bw/day. Hypertrophy of the thyroid was reported in animals of either sex treated with 500 mg/kg bw/day, and for males treated with 150 mg/kg bw/day.

In a follow-up study to evaluate the hepatic effects and the slight increase in bilirubin seen at the lowest dose of 15 mg/kg bw/day in the previous study, 2-pentylthiophene was administered by gavage to one group of five male and five female Sprague-Dawley Crl:CD® (SD) IGS BR strain rats, for twenty-eight consecutive days, at a dose level of 3 mg/kg/day. A control group of five males and five females was given vehicle alone (Arachis oil BP). Clinical signs, body weight development and food and water consumption monitored during the study revealed no significant differences between test

and control animals. Haematology and blood chemistry were evaluated for all animals at the end of the study and showed no significant changes. There were no treatment-related changes in spleen weights for treated animals in comparison to controls. No macroscopic abnormalities were detected at necropsy.

The authors concluded that oral administration of 2-pentylthiophene to rats for a period of up to twenty eight days at a dose level of 3 mg/kg bw/day resulted in no toxicologically or haematologically significant effects, The NOAEL was considered to be 3 mg/kg bw/day (Marr and Watson, 2007). The Panel agrees with this NOAEL.

The candidate substance thiophene-2-carbaldehyde [FL-no: 15.107] was tested in a subacute toxicity test, in which groups of 5 rats (sex not specified) were exposed to 0, 95 and 840 mg/kg bw/day for 11 days (Sharp, 1979). No adverse signs or changes in behaviour were noted in treated animals. The reported NOAEL was 95 mg/kg bw/day**.**

Data are also available from short-term tests on two supporting substances in this subgroup A-Ib 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] and 3-acetyl-2,5-dimethylthiophene [FL-no: 15.024], administered to male and female rats for 14 days at 10 mg/kg bw/day (Gill and Van Miller, 1987b). Both substances produced minor body weight changes in both sexes and organ weight changes in males only.

The Panel considered that the studies available on the candidate substance in this subgroup, 2-Pentylthiopene (Register name: *sec*-pentylthiophene) [FL-no: 15.096] and thiophene-2-carbaldehyde [FL-no: 15.107], together with the two studies on supporting substances, were inadequate for deriving an appropriate NOAEL to be used in the Procedure because of the short durations of these studies. According to the practice of the Panel, a minimum requirement to provide an adequate NOAEL for flavourings in the Procedure is a 90-day study.

Subgroup A-Ic: Thiophenes with thiol-containing ring substituents

No toxicity data were available for the three candidate substances in this subgroup [FL-no: 15.082, 15.087 and 15.108]. The supporting substance 2-thienyl disulfide (2,2'-dithiodithiophene) [FL-no: 15.008] was tested in a 90-day dietary feeding study in male and female rats at a single dose level of 0.29 mg/kg bw/day (Morgareidge and Oser, 1970g). Body weight changes, food consumption, haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed, and accordingly a NOAEL of 0.29 mg/kg bw/day could be established.

Subgroup A-II: Thiazoles

There were no subacute, subchronic or chronic toxicity studies on any of the 23 candidate thiazoles in subgroup A-II.

A 90-day dietary feeding study was carried out on the supporting substance 5-acetyl-2,4-dimethylthiazole [FL-no: 15.011] in male (23) and female (23) rats, at a single dose level of approximately 25 mg/kg bw/day (Shellenberger, 1971d). Body weight, food consumption, haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed and accordingly a NOAEL of 25 mg/kg bw/day could be established.

In a 90-day study on the supporting substance 2-acetylthiazole [FL-no: 15.020], male rats (10/group) were fed diets containing 0, 100, 1000 or 10,000 mg/kg test substance, reported to be equivalent to an average daily intake of 0, 5, 50 and 500 mg/kg bw/day (Wheldon et al., 1970). The dose level in the highest dose group was increased to 20,000 mg/kg at week 6. Test substance-related changes at 10,000 - 20,000 mg/kg included reduced body weight gain, increased relative liver, adrenal and thyroid weights and minimal fatty changes in the liver. The authors reported a NOAEL of 50 mg/kg bw/day.

However, due to limitations in the study design and reporting, the Panel concluded that this NOAEL could not be used in the Procedure.

Two further 90-day studies were carried out at single dose levels with the supporting substances 2-methyl-5-methoxythiazole [FL-no: 15.002] and 2,4-dimethyl-5-vinylthiazole [FL-no: 15.005] (Posternak et al., 1975). Due to limitations in reporting of experimental details and results, the Panel concluded that these studies could not be used to derive a NOAEL to be used in the Procedure.

Oral intakes of up to 500 mg thiamine/day (8 mg/kg bw/day in a 60 kg individual) have been reported to have no adverse effects in humans (SCF, 2001).

Subgroup A-III: Benzothiazoles

There were no toxicological studies available on the candidate substance 2-methyl-4,5-benzothiazole [FL-no: 15.088] in subgroup A-III. The Panel concluded that data on the supporting substance benzothiazole [FL-no: 15.016] could not be used in the Procedure for 2-methyl-4,5-benzothiazole, since the unsubstituted benzothiazole is anticipated to be metabolised differently from the substituted benzothiazole in subgroup A-III (see Section 4 and Annex III).

Subgroup B-I: Dihydrothiophenes

There were no toxicological data available on the candidate substance 4-hydroxy-2,5-dimethylthiophen-3(2H)-one [FL-no: 15.077] nor were there any data on supporting substances for subgroup B-I.

Subgroup B-II: Thiazolines

There were no toxicological data available for the three candidate substances in this subgroup. The supporting substance 2-(sec-butyl)-4,5-dimethyl-3-thiazoline [FL-no: 15.029] was tested in a 90-day dietary feeding study in male and female rats (15/sex) at one dose level of approximately 1 mg/kg bw/day (Babish and Re, 1978). Body weight changes, food consumption, limited haematological and clinical chemistry parameters were assessed, urinalysis was undertaken and a comprehensive range of tissues were examined histopathologically. No changes were detected in any of the parameters assessed and accordingly a NOAEL of 1 mg/kg bw/day could be established.

Subgroup B-III: Thiazolidines

There were no toxicological data available on the candidate substances 2-methyl thiazolidine and 2-propyl thiazolidine [FL-no: 15.090 and 15.099] nor were there any data on structurally related substances for subgroup B-III.

Subgroup B-IV: Dithiazines

There were no toxicological data available on the six candidate substances in subgroup B-IV [FL-no: 15.042, 15.054, 15.055 15.057, 15.079 and 15.135]. Short-term toxicity tests are available on the mixture 2-isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (Rush, 1989a) and the mixture 2-isopropyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (Rush, 1989b). The mixtures were incorporated in the diet of Sprague-Dawley rats (5/sex/group) intake of approximately 10 mg/kg bw/day over a period of 14 days. No treatment-related effects were seen. The Panel considered that these studies were inadequate for deriving a NOAEL to be used in the Procedure, because of the short duration of the studies.

Subgroup B-V: Dihydrothiazines

There were no toxicological data available on the candidate substances 6-acetyl-2,3-dihdro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) or 5-acetyl-2,3-dihdro-1,4-thiazine [FL-no: 15.133] nor were there any data on supporting substances for subgroup B-V.

Subgroup B-VI: Thiadiazines

No toxicological data were available on tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine [FL-no: 15.129] or on structurally related supporting substances.

The repeated dose toxicity studies are summarised in Annex IV, Table IV.2.

8.3. Developmental / Reproductive Toxicity Studies

As described in Section 8.2, a combined repeat dose/reproductive and developmental toxicity study, in accordance with OECD Test Guideline 422, has been carried out with thiophene, in which males were dosed for 42 days and females from 14 days before mating until day 3 of lactation (Nagao, 2006). The full report of this study was in Japanese, however a summary of the study design, results and conclusions to be drawn were available to the Panel in English.

Groups of Sprague-Dawley Rats (13/sex/dose) were administered thiophene via gavage at dose levels of 0, 25, 100, or 400 mg/kg bw/day. No adverse effects on copulation, ovulation, or fertility in treatment groups were observed when compared to the control groups. However, in each group, abnormal parturition was found. Females in the 100 or 400 mg/kg bw/day group, showing evidence of histopathological change in the cerebellum, exhibited abnormal lactation. Pups born to the 400 mg/kg bw/day group showed reduced birth weights and viability decreased at postnatal day 4. No morphological abnormalities associated with the administration of thiophene were found in any pup. With respect to effects on reproduction, the NOAEL was suggested by the authors to be 400 mg/kg bw/day for males and 25 mg/kg bw/day for females (Nagao, 2006).

No other developmental toxicity or reproductive toxicity studies were available for any of the 56 candidate substances or for any of the 29 supporting substances evaluated by the JECFA.

The developmental/reproductive toxicity study is summarised in Annex IV, Table IV.3.

8.4. Genotoxicity Studies

Genotoxicity data were provided for 12 of the candidate substances. These 12 substances belong to subgroup A-Ia: thiophene [FL-no: 15.106]; subgroup A-Ib: 2-methylthiophene [FL-no: 15.091], 3-methylthiophene [FL-no: 15.092], 2,5-dimethylthiophene [FL-no: 15.064], 2-acetylthiophene [FL-no: 15.040], 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107], 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074]; subgroup A-II: 2,4-dimethylthiazole [FL-no: 15.062]; subgroup A-III: 2-methyl-4,5-benzothiazole [FL-no: 15.088]; subgroup B-III: 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099]. There were also mutagenicity data on four supporting substances and on four other structurally related substances. All available information on genotoxicity of the 12 candidate and the four supporting substances and of four other structurally related substances is based upon *in vitro* studies only**.**

Subgroup A-I:

Thiophene [FL-no: 15.106], 2-methylthiophene [FL-no: 15.091], 3-methyl-thiophene [FL-no: 15.092] and 2,5-dimethylthiophene [FL-no: 15.064] were reported to be negative in microbial mutagenicity assays. 2-Acetylthiophene [FL-no: 15.040] was negative in microbial tests, using *Salmonella typhimurium strains* TA98 and TA100, with and without metabolic activation and in the SOS chromotest with metabolic activation. 2-Acetylthiophene was reported to be positive without

metabolic activation in the SOS *Escherichia coli* chromotest (Mosier et al., 2003). In the same study, 2-acetyl-3-methylthiophene [FL-no: 15.037], thiophene-2-carbaldehyde [FL-no: 15.107] and 5-ethylthiophene-2-carbaldehyde [FL-no: 15.074] gave positive results without metabolic activation in the SOS *E. coli* chromotest. The concentrations tested were not reported for any of the substances subjected to the SOS *E. coli* chromotest (Mosier et al., 2003). The Panel considered the endpoint of this test inappropriate for the estimation of genotoxic potential. The supporting substance 5-methyl-2-thiophenecarbaldehyde [FL-no: 15.004] was negative in a microbial mutagenicity assay.

Thiophene was tested in accordance to OECD guidelines in a bacterial reverse mutation test in strains of *S. typhimurium* and in strain WP2 uvrA of *E. coli*. No evidence of mutagenic response was reported when strains TA100, TA1535, TA98, and TA1537 of *S. typhimurium* were incubated at concentrations of 0, 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/plate with and without S9 metabolic activation. Toxicity was observed at 1250 µg/plate in TA1537, and 2500 µg/plate in strains TA100, TA1535 and TA98 also with and without metabolic activation. Toxicity was observed at 5000 µg/plate in WP2 with and without S9 metabolic activation (Shibuya, 2006).

In a chromosomal aberration test, thiophene was tested on Chinese hamster lung cells in accordance with Japanese Guidelines. No chromosomal aberrations or polyploidy was reported when incubated with concentrations of 0, 210, 420, 840 µg/mL of thiophene, with and without metabolic activity (Tanaka, 2006).

Subgroup A-II:

2,4-Dimethylthiazole [FL-no: 15.062] was reported to be negative in microbial assays, using *S. typhimurium,* but only in strain TA100 and only in the absence of metabolic activation (Voogd et al., 1983). Two supporting substances, 4,5-dimethylthiazole [FL-no: 15.017] and 4-methylthiazole [FL-no: 15.035] were negative in microbial mutagenicity assays.

Subgroup A-III:

2-Methyl-4,5-benzothiazole [FL-no: 15.088] was reported to be negative in an Ames test but only a summary report was available (Longfellow, 1998a). The supporting substance benzothiazole [FL-no: 15.016] was negative in microbial mutagenicity assay and in the mouse lymphoma test.

Subgroups B-I and B-II:

No genotoxicity information was available for any candidate or supporting substances in these subgroups. However, considering the structural similarities between the thiazolines in subgroup B-II and the thiazolidines in subgroup B-III, the Panel also concluded that the thiazolines [FL-no: 15.060, 15.086 and 15.119] could not be evaluated through the Procedure (see Subgroup B-III below).

Subgroup B-III:

The two candidate substances 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] as well as the structurally related ethyl, isopropyl, n-butyl and isobutyl thiazolidine have all been reported to be positive in the Ames tests (TA98 and TA100) (Mihara and Shibamoto, 1980). Owing to limited reporting, the data could not be properly evaluated. Nevertheless, these reports do raise the possibility of a genotoxic potential of these thiazolidines. Accordingly, it was concluded not to evaluate the candidate substances 2-methylthiazolidine and 2-propylthiazolidine through the Procedure.

Subgroup B-IV:

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

Subgroup B-V:

The two candidate substances 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133] are alpha,beta unsaturated ketones i.e. they have a structural alert for genotoxicity (EFSA, 2008b) and as there are no genotoxicity data available a concern for genotoxicity cannot be ruled out.

Subgroup B-VI:

No genotoxicity information was available for any candidate or supporting substance in this subgroup.

Overall conclusion on genotoxicity:

It is concluded that the genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in the present revision of FGE.21, Revision 3. However, except for the two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], the two thiazolidines 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and the three structurally related thiazolines 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 49 candidate substances using the Procedure.

9. Conclusions

All candidate substances are five- or six-member sulphur-containing heterocyclic compounds, some of which also contain nitrogen. They belong to chemical groups 29 and 30 and they can be divided into two main groups, (A) those with an aromatic ring (44 candidate substances) and (B) those with a non-aromatic ring structure (15 candidate substances). Except for thiophene, all are ring-substituted with one or more of the following substituents: alkyl, alkenyl, aryl, alcohol, keto, thio. For assessment purposes, the following further subdivision of groups (A) and (B) has been made:

Group (A): Aromatic:

Subgroup A-Ia: Thiophene.

Subgroup A-Ib: Thiophene derivatives with non-thiol-containing ring substituents.

Subgroup A-Ic: Thiophene derivatives with thiol-containing ring substituents.

Subgroup A-II: Thiazole derivatives.

Subgroup A-III: Benzothiazoles.

Group (B): Non aromatic:

Subgroup B-I: Dihydrothiophenes.

Subgroup B-II: Thiazolines.

Subgroup B-III: Thiazolidines.

Subgroup B-IV: Dithiazine derivatives.

Subgroup B-V: Dihydrothiazines.

Subgroup B-VI: Thiadiazine derivatives.

Twelve candidate substances possess one or more chiral centres. For four of these substances [FL-no: 15.042, 15.057, 15.079 and 15.135] the stereoisomeric composition has not been specified sufficiently.

Forty-six of the candidate substances belong to structural class II and 13 belong to structural class III.

Forty-four of the candidate substances have been reported to occur naturally in a wide range of foods.

According to the default MSDI approach, the flavouring substances in this group have intakes in Europe ranging from 0.0012 to 5.7 microgram/capita/day, which are below the thresholds of concern values for structural class II (540 microgram/person/day) and structural class III (90 microgram/person/day) substances.

On the basis of the reported annual production volumes of the candidate substances as flavourings in Europe, the combined estimated daily *per capita* intakes, per subgroup and structural class range from 0.14 to 8.0 microgram. For none of the subgroups do the total combined intakes exceed the thresholds of concern of 540 microgram/person/day or of 90 microgram/person/day for structural class II and III, respectively.

The candidate substances are structurally related to 29 supporting substances evaluated by the JEFCA at its 59th JECFA meeting (JECFA, 2002c). The total combined daily per capita intakes (in Europe) of candidate and supporting substances in each of the four subgroups for which supporting substances were available are: subgroup A-Ib (18 substances from class II) 22 microgram; subgroup A-Ic (five substances from class III) 0.15 microgram; subgroup A-II (36 substances from class II) 500 microgram and subgroup A-III (two substances from class III) 1.2 microgram. For none of the subgroups do the combined intakes exceed the thresholds of concern for compounds belonging to structural class II of 540 microgram/person/day or to structural class III of 90 microgram/person/day.

It is concluded that the genotoxicity data are limited and that genotoxicity could not be assessed adequately for the flavouring substances in FGE.21Rev3. However, except for the two dihydrothiazines, 6-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.114] (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine) and 5-acetyl-2,3-dihydro-1,4-thiazine [FL-no: 15.133], two thiazolidines, 2-methylthiazolidine [FL-no: 15.090] and 2-propylthiazolidine [FL-no: 15.099] and the three structurally related thiazolines, 2-methyl-2-thiazoline [FL-no: 15.086], 2,4-dimethyl-3-thiazoline [FL-no: 15.060] and 2-isobutyl-3-thiazoline [FL-no: 15.119], the genotoxicity data available do not preclude the evaluation of the remaining 52 candidate substances using the Procedure.

The metabolism data available were insufficient to allow conclusions about the metabolic fate of the candidate substances, and accordingly the 52 candidate substances evaluated through the Procedure could not be anticipated to be metabolised to innocuous products. However, the Panel concluded that the evidence of binding to macromolecules from possible formation of electrophilic metabolites, (e.g. by either ring scission or S-oxidation) was not sufficiently strong to preclude the application of the Procedure.

Toxicological data on both candidate and supporting substances were limited and did not include information about chronic or reproductive toxicity, with the exception of a combined repeat dose and reproduction and developmental toxicity study on thiophene [FL-no: 15.106]. Valid toxicological data which could provide an adequate margin of safety compared to the intakes from use as flavouring substances were only available for the 26 candidate substances from subgroup A-Ic (thiophenes with thiol-containing ring substituents) and subgroup A-II (thiazoles).

For the remaining 26 candidate substances belonging to the subgroups of thiophene itself (A-Ia), thiophenes with non-thiol-containing ring substituents (A-Ib), benzothiazoles (A-III), dihydrothiophenes (B-I), dithiazines (B-IV) and thiadiazines (B-VI), the Panel concluded that there were insufficient data available to provide margins of safety from their use as flavouring substances and that additional toxicity data are needed.

It is considered that 26 candidate substances evaluated through the Procedure [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118] are not of safety concern at their estimated levels of intake based on the MSDI approach, whereas for 26 candidate substances [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.057, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.079, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107, 15.129 and 15.135], additional toxicological data are required.

The estimated intakes, based on the mTAMDI, for the 41 candidate substances assigned to structural class II and evaluated using the Procedure, range from 78 to 4000 microgram/person/day. For one of these substances, [FL-no: 15.129], the mTAMDI value of 4000 microgram/person/day is above the threshold of concern of 540 microgram/person/day for structural class II. The estimated intakes based on the mTAMDI of the nine candidate substances assigned to structural class III and evaluated through the Procedure ranged from 78 to 250 microgram/person/day. For four of the candidate substances, [FL-no: 15.042, 15.055 15.088 and 15.135] the estimated intakes are above the threshold of concern for structural class III substances of 90 microgram/person/day. For two candidate substances, [FL-no: 15.057 and 15.079], assigned to structural class III, no use levels were provided at all. Therefore, more reliable exposure data are required for [FL-no: 15.042, 15.055, 15.057, 15.079, 15.088, 15.129 and 15.135]. On the basis of such additional data, these flavouring substances should be re-evaluated using the Procedure

In order to determine whether the conclusion for the 52 candidate substances which have been evaluated using the Procedure can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications including purity and identity for the materials of commerce have been provided for 47 flavouring substances evaluated through the Procedure. Information on stereoisomerism is incomplete for four of the substances, [FL-no: 15.042, 15.057, 15.079 and 15.135] and for one substance, [FL-no: 15.129], an identity test is missing.

Thus, the final evaluation of the materials of commerce cannot be performed for the five substances [FL-no: 15.042, 15.057, 15.079, 15.129 and 15.135], pending further information on stereoisomerism and/or identity test.

For 26 flavouring substances evaluated through the Procedure [FL-no: 15.037, 15.040, 15.042, 15.043, 15.045, 15.054, 15.055, 15.057, 15.064, 15.070, 15.072, 15.074, 15.076, 15.077, 15.079, 15.088, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106, 15.107, 15.129 and 15.135] the Panel considered that additional toxicity data are needed. Furthermore, for seven substances [FL-no: 15.060, 15.086, 15.090, 15.099, 15.114, 15.119 and 15.133] the Panel concluded that additional genotoxicity data are required.

For the remaining 26 flavouring substances [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.082, 15.084, 15.085, 15.087, 15.089, 15.098, 15.108, 15.115, 15.116 and 15.118], evaluated using the Procedure, the Panel concluded that they would present no safety concern at their estimated levels of intake based on the MSDI approach.

TABLE 1: SPECIFICATION SUMMARY OF THE SUBSTANCES IN FGE 21REV3

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, Revision 3

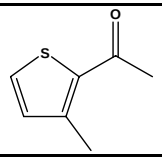
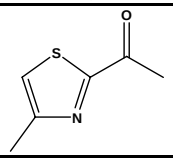
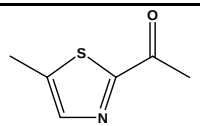
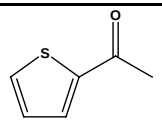
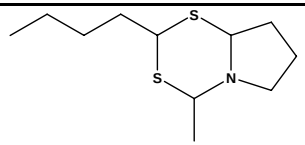
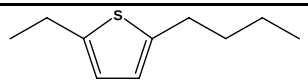
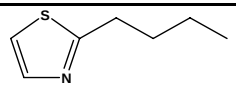
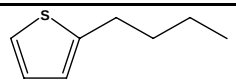
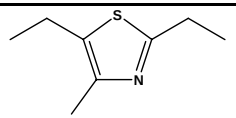
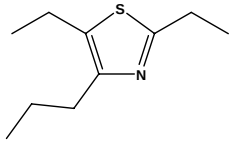
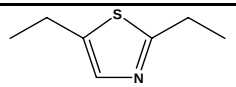
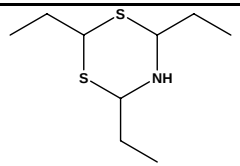
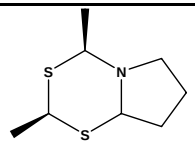
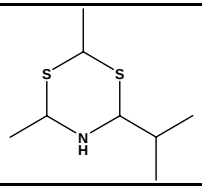
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.037	2-Acetyl-3-methylthiophene		11590 13679-72-6	Liquid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	216 MS 95 %	1.558-1.564 1.130-1.136	Substance not supported by Industry (EFFA, 2009c).
15.038	2-Acetyl-4-methylthiazole		11589 7533-07-5	Solid C_6H_7NOS 141.19	Practically insoluble or insoluble Freely soluble	95 (16 hPa) 34 MS 95 %	n.a. n.a.	
15.039	2-Acetyl-5-methylthiazole		59303-17-2	Solid C_6H_7NOS 141.19	Practically insoluble or insoluble Freely soluble	93 (16 hPa) 30 NMR 95 %	n.a. n.a.	
15.040	2-Acetylthiophene		11728 88-15-3	Solid C_6H_6OS 126.17	Practically insoluble or insoluble Freely soluble	213 34 MS 98 %	1.563-1.569 1.164-1.171	
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine		132344-97-9	Solid $C_{11}H_{21}NS_2$ 231.42	Practically insoluble or insoluble Freely soluble	304 164 NMR 95 %	n.a. n.a.	Mixture of diastereoisomers (EFFA, 2010a). Composition of stereoisomeric mixture to be specified. CASrn in Register does not specify stereo-isomeric composition. Substance not supported by Industry (EFFA, 2009c).
15.043	2-Butyl-5-ethylthiophene		11596 54411-06-2	Solid $C_{10}H_{16}S$ 168.30	Practically insoluble or insoluble Freely soluble	231 54 MS 95 %	n.a. n.a.	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, Revision 3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.044	2-Butylthiazole		 11597 37645-61-7	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	212 79 NMR 95 %	n.a. n.a.	
15.045	2-Butylthiophene		 1455-20-5	Liquid $C_8H_{12}S$ 140.24	Practically insoluble or insoluble Freely soluble	182 MS 95 %	1.504-1.510 0.951-0.957	
15.050	2,5-Diethyl-4-methylthiazole		 41981-71-9	Solid $C_8H_{13}NS$ 155.26	Practically insoluble or insoluble Freely soluble	85 (20 hPa) 115 MS 95 %	n.a. n.a.	
15.051	2,5-Diethyl-4-propylthiazole		 4276-68-0	Solid $C_{10}H_{17}NS$ 183.31	Practically insoluble or insoluble Freely soluble	72 (21 hPa) 139 NMR 95 %	n.a. n.a.	
15.052	2,5-Diethylthiazole		 15729-76-7	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	187 92 MS 95 %	n.a. n.a.	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine		 54717-17-8	Solid $C_9H_{19}NS_2$ 205.38	Practically insoluble or insoluble Freely soluble	287 188 MS 95 %	n.a. n.a.	Mixture of diastereoisomers (EFFA, 2010a). Mixture of isomers ((R/R), (R/S), (S/R) & (S/S) at equal ratio, i.e. 25 % of each) (EFFA, 2011f).
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine		4321 116505-60-3	Solid $C_8H_{15}NS_2$ 189.34	Practically insoluble or insoluble Freely soluble	235 130 MS 95 %	n.a. n.a.	Register name to be changed to [2S-(2α,4α,8αβ)] 2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine (EFFA, 2010a).
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine 6)		3782 104691-40-9	Liquid $C_8H_{17}NS_2$ 191.36	Slightly soluble Soluble	109 (0.23hPa) MS 71 %	1.496-1.500 0.951-0.959	At least 44 % 2-Isopropyl-4,6-dimethyl and 27 % 4-Isopropyl-2,6-dimethyl; sec comp. at least 24 % 2,4,6-Trimethyldihydro--; 6-Methyl-2,4-diisopropyl-; 4-Methyl-2,6-diisopropyl-

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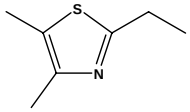
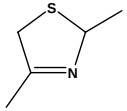
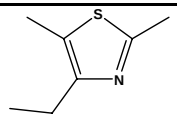
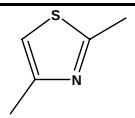
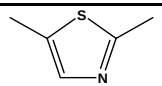
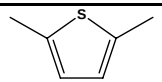
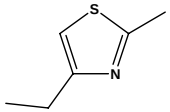
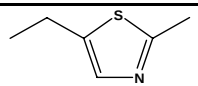
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
								, 2,4,6-Triisopropyl-dihydro-1,3,5-dithiazine (EFFA, 2011h).
15.058	4,5-Dimethyl-2-ethylthiazole		 873-64-3	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	185 104 MS 96 %	n.a. n.a.	
15.060	2,4-Dimethyl-3-thiazoline		 60755-05-7	Solid C_5H_9NS 115.19	Practically insoluble or insoluble Freely soluble	52 (15 hPa) 102 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010a).
15.061	2,5-Dimethyl-4-ethylthiazole		 32272-57-4	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	185 104 MS 95 %	n.a. n.a.	
15.062	2,4-Dimethylthiazole		 11605 541-58-2	Solid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	145 69 MS 95 %	n.a. n.a.	
15.063	2,5-Dimethylthiazole		 4175-66-0	Solid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	152 69 MS 95 %	n.a. n.a.	
15.064	2,5-Dimethylthiophene		 11609 638-02-8	Liquid C_6H_8S 112.19	Practically insoluble or insoluble Freely soluble	135 MS 95 %	1.508-1.514 0.982-0.988	
15.067	4-Ethyl-2-methylthiazole		 32272-48-3	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	91 (89 hPa) 80 MS 95 %	n.a. n.a.	
15.068	5-Ethyl-2-methylthiazole		 19961-52-5	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	170 80 MS 95 %	n.a. n.a.	

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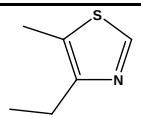
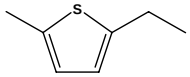
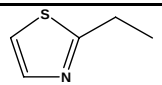
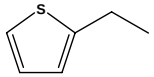
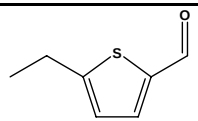
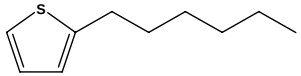
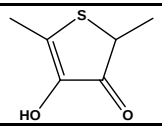
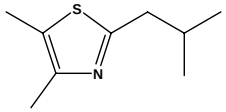
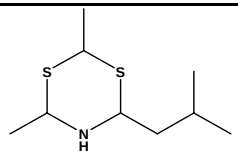
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.069	4-Ethyl-5-methylthiazole		 52414-91-2	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	174 80 MS 95 %	n.a. n.a.	
15.070	2-Ethyl-5-methylthiophene		 40323-88-4	Liquid $C_7H_{10}S$ 126.22	Practically insoluble or insoluble Freely soluble	158 MS 97 %	1.503-1.509 0.963-0.970	
15.071	2-Ethylthiazole		 15679-09-1	Liquid C_5H_7NS 113.18	Practically insoluble or insoluble Freely soluble	148 MS 95 %	1.511-1.517 1.058-1.064	
15.072	2-Ethylthiophene		 11614 872-55-9	Liquid C_6H_8S 112.19	Practically insoluble or insoluble Freely soluble	133 MS 95 %	1.510-1.516 0.989-0.995	
15.074	5-Ethylthiophene-2-carbaldehyde		 36880-33-8	Solid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	104 (12 hPa) 62 MS 95 %	n.a. n.a.	
15.076	2-Hexylthiophene		4137 11616 18794-77-9	Solid $C_{10}H_{16}S$ 168.30	Practically insoluble or insoluble Freely soluble	92 (13 hPa) 41 MS 95 %	n.a. n.a.	
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		 26494-10-0	Solid $C_6H_8O_2S$ 144.19	Practically insoluble or insoluble Freely soluble	296 133 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010a). Substance not supported by Industry (EFFA, 2009c).
15.078	2-Isobutyl-4,5-dimethylthiazole		 11617 53498-32-1	Solid $C_9H_{15}NS$ 169.29	Practically insoluble or insoluble Freely soluble	267 112 MS 97 %	n.a. n.a.	
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine 6)		3781 101517-87-7	Liquid $C_9H_{19}NS_2$ 205.39	Slightly soluble Soluble	115 (0.33 hPa) MS 82 %	1.488-1.492 0.961-0.967	At least 64 % 2-isobutyl-4,6-dimethyl and 18 % 4-Isobutyl-2,6-dimethyl; sec. components at least 13 % 2,4,6-trimethyl-; 2,4-diisobutyl-6-methyl-; 2,6-dimethyl-4-butyldihydro-1,3,5-

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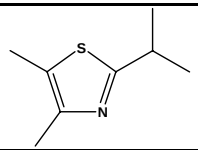
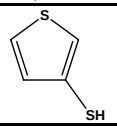
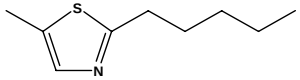
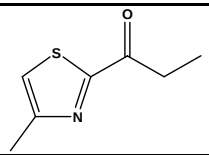
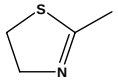
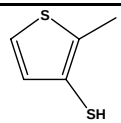
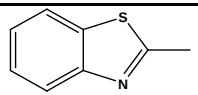
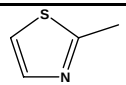
								dithiazine; substituted 1,3,5-thiadiazine.(EFFA, 2011h).
15.080	2-Isopropyl-4,5-dimethylthiazole		53498-30-9	Solid $C_8H_{13}NS$ 155.26	Practically insoluble or insoluble Freely soluble	244 101 MS 95 %	n.a. n.a.	
15.082	3-Mercaptothiophene		7774-73-4	Liquid $C_4H_4S_2$ 116.20	Slightly soluble Freely soluble	171 MS 95 %	1.617-1.623 1.248-1.254	
15.084	5-Methyl-2-pentylthiazole		86290-21-3	Solid $C_9H_{15}NS$ 169.26	Practically insoluble or insoluble Freely soluble	262 114 NMR 95 %	n.a. n.a.	
15.085	4-Methyl-2-propionylthiazole		11622 13679-83-9	Solid C_7H_9NOS 155.21	Practically insoluble or insoluble Freely soluble	86 (12 hPa) 142 NMR 95 %	n.a. n.a.	
15.086	2-Methyl-2-thiazoline		2346-00-1	Solid C_4H_7NS 101.17	Slightly soluble Freely soluble	144 62 MS 95 %	n.a. n.a.	
15.087	2-Methyl-3-mercaptothiophene		2527-76-6	Solid $C_5H_6S_2$ 130.22	Slightly soluble Freely soluble	73 (15 hPa) 27 MS 95 %	n.a. n.a.	
15.088	2-Methyl-4,5-benzothiazole		120-75-2	Liquid C_8H_7NS 149.21	Practically insoluble or insoluble Freely soluble	238 MS 95 %	1.612-1.618 1.173-1.179	Substance not supported by Industry (EFFA, 2009c).
15.089	2-Methylthiazole		11626 3581-87-1	Liquid C_4H_5NS 99.15	Slightly soluble Freely soluble	128 MS 95 %	1.511-1.517 1.109-1.116	

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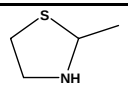
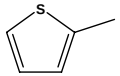
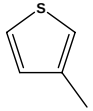
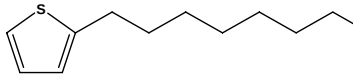
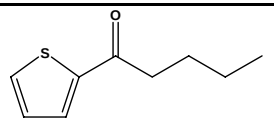
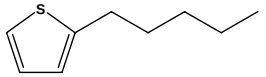
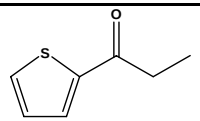
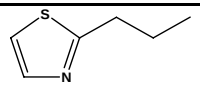
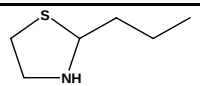
15.090	2-Methylthiazolidine		 24050-16-6	Liquid C_4H_9NS 103.18	Slightly soluble Freely soluble	160 MS 95 %	1.522-1.528 1.519-1.525	Racemate (EFFA, 2010a). Substance not supported by Industry (EFFA, 2009c).
15.091	2-Methylthiophene		 11631 554-14-3	Liquid C_5H_6S 98.16	Practically insoluble or insoluble Freely soluble	112 MS 97 %	1.516-1.523 1.013-1.022	
15.092	3-Methylthiophene		 11632 616-44-4	Liquid C_5H_6S 98.16	Practically insoluble or insoluble Freely soluble	115 MS 95 %	1.514-1.520 1.018-1.024	
15.093	2-Octylthiophene		 880-36-4	Solid $C_{12}H_{20}S$ 196.35	Practically insoluble or insoluble Freely soluble	259 64 MS 95 %	n.a. n.a.	
15.094	2-Pentanoylthiophene		 53119-25-8	Solid $C_9H_{12}OS$ 168.26	Practically insoluble or insoluble Freely soluble	257 80 MS 95 %	n.a. n.a.	Substance not supported by Industry (EFFA, 2009c).
15.096	sec-Pentylthiophene		4387 11634 4861-58-9	Liquid $C_9H_{14}S$ 154.27	Practically insoluble or insoluble Freely soluble	201 MS 95 %	1.495-1.501 0.940-0.946	Register name to be changed to 2-Pentylthiophene (EFFA).
15.097	2-Propionylthiophene		 11635 13679-75-9	Solid C_7H_8OS 140.20	Practically insoluble or insoluble Freely soluble	225 57 MS 95 %	n.a. n.a.	
15.098	2-Propylthiazole		 17626-75-4	Solid C_6H_9NS 127.20	Practically insoluble or insoluble Freely soluble	172 78 MS 95 %	n.a. n.a.	
15.099	2-Propylthiazolidine		 24050-10-0	Solid $C_6H_{13}NS$ 131.24	Practically insoluble or insoluble Freely soluble	75 (13 hPa) 84 MS 95 %	n.a. n.a.	Racemate (EFFA, 2010a). Substance not supported by Industry (EFFA, 2009c).
15.106	Thiophene		 11647 110-02-1	Liquid C_4H_4S 84.14	Practically insoluble or insoluble Freely soluble	84 MS 95 %	1.525-1.531 1.062-1.068	

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, Revision 3

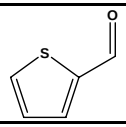
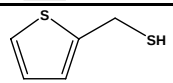
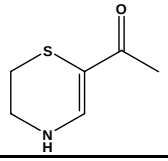
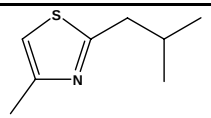
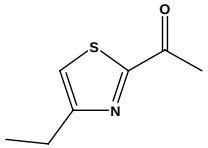
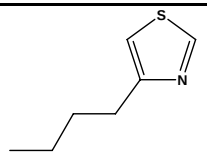
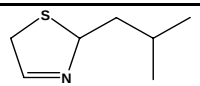
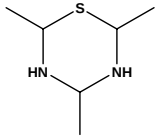
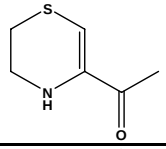
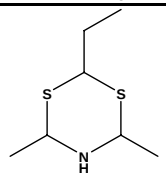
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
15.107	Thiophene-2-carbaldehyde		11874 98-03-3	Liquid C_5H_4OS 112.15	Slightly soluble Freely soluble	198 MS 95 %	1.585-1.592 1.216-1.225	Substance not supported by Industry (EFFA, 2009c).
15.108	2-Thiophenemethanethiol		6258-63-5	Liquid $C_5H_6S_2$ 130.22	Slightly soluble Freely soluble	82 (16 hPa) MS 95 %	1.571-1.578 1.165-1.171	
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine		4296 101417-25-8	Solid C_6H_9NOS 143.2	Sparringly soluble Freely soluble	242 126 NMR 95 %	n.a. n.a.	Register name to be changed to 6-Acetyl-2,3-dihydro-1,4-thiazine. Substance not supported by Industry (EFFA, 2009c).
15.115	2-Isobutyl-4-methyl thiazole		61323-24-8	Solid $C_8H_{13}NS$ 155.26	Slightly soluble Freely soluble	189 88 MS 95 %	n.a. n.a.	Register name to be changed to 2-Isobutyl-4-methylthiazole.
15.116	2-Acetyl-4-ethylthiazole		233665-91-3	Solid C_7H_9NOS 155.22	Slightly soluble Freely soluble	270 142 NMR 95 %	n.a. n.a.	
15.118	4-Butylthiazole		53833-33-3	Solid $C_7H_{11}NS$ 141.23	Practically insoluble or insoluble Freely soluble	212 79 MS 95 %	n.a. n.a.	
15.119	2-Isobutyl-3-thiazoline		39800-92-5	Solid $C_7H_{13}NS$ 143.25	Very slightly soluble Freely soluble	197 80 MS 95 %	n.a. n.a.	Racemate (EFFA, 2011f).
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		53897-63-5	Solid $C_6H_{14}N_2S$ 146	Soluble Soluble	216-266 (1 hPa) 65-70 99 %	n.a. n.a.	ID 7). Mixture of diastereoisomers (EFFA, 2010a). Mixture of isomers ((R/R), (R/S), (S/R) & (S/S) at equal

Table 1: Specification Summary of the Substances in the Flavouring Group Evaluation 21, Revision 3

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	Specification comments
								ratio, i.e. 25 % of each) (EFFA, 2011f). Substance not supported by Industry (EFFA, 2009c).
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine		 164524-93-0	Solid C_6H_9NOS 143	Soluble Soluble	289+/-30 67-69 98%	n.a. n.a.	ID 7). Substance not supported by Industry (EFFA, 2009c).
15.135	Ethyl thialdine		4667 54717-14-5	Liquid $C_7H_{15}NS_2$ 177.33	Poorly soluble Soluble	75 IR NMR MS 90 %	1.5344-1.5544 1.0745-1-0765	CASrn in Register does not specify stereoisomeric composition. Min assay value 90 %. Remaining impurities: 3,5-diethyl-1,2,4-trithiolane (<5 %) , thialdine (<2 %), other impurities (<3 %) (Flavour Industry, 2011f). Composition of stereoisomeric mixture to be specified.

1) Solubility in water, if not otherwise stated.
2) Solubility in 95 % ethanol, if not otherwise stated.
3) At 1013.25 hPa, if not otherwise stated.
4) At 20°C, if not otherwise stated.
5) At 25°C, if not otherwise stated.
6) Stereoisomeric composition not specified.
7) ID: Missing identification test.

TABLE 2: SUMMARY OF SAFETY EVALUATION APPLYING THE PROCEDURE (BASED ON THE MSDI APPROACH)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

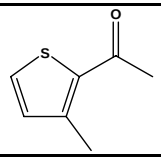
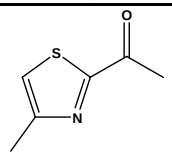
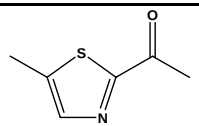
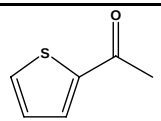
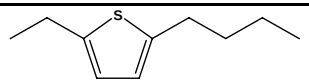
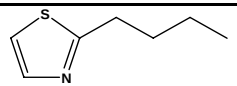
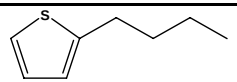
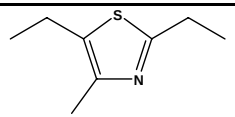
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.037	2-Acetyl-3-methylthiophene		0.18	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.038	2-Acetyl-4-methylthiazole		0.0049	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.039	2-Acetyl-5-methylthiazole		0.0024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.040	2-Acetylthiophene		2.2	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.043	2-Butyl-5-ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.044	2-Butylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.045	2-Butylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.050	2,5-Diethyl-4-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

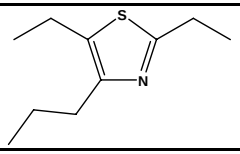
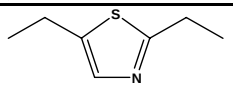
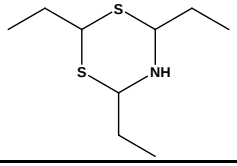
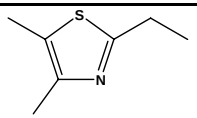
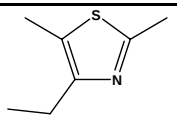
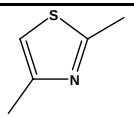
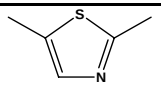
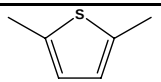
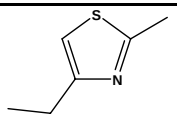
FL-no	EU Register name	Structural formula	MSDI 1) (μg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.051	2,5-Diethyl-4-propylthiazole		0.0012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.052	2,5-Diethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.058	4,5-Dimethyl-2-ethylthiazole		0.015	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.061	2,5-Dimethyl-4-ethylthiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.062	2,4-Dimethylthiazole		0.61	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.063	2,5-Dimethylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.064	2,5-Dimethylthiophene		0.23	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.067	4-Ethyl-2-methylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

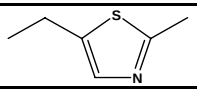
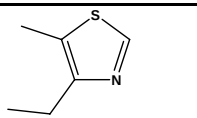
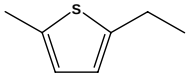
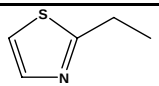
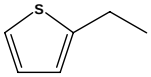
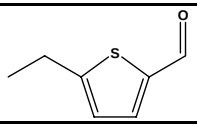
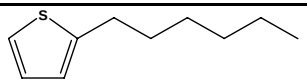
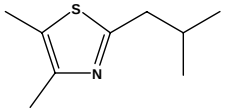
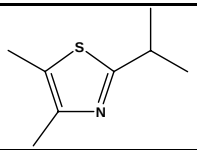
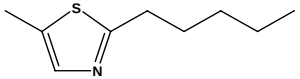
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.068	5-Ethyl-2-methylthiazole		0.0061	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.069	4-Ethyl-5-methylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.070	2-Ethyl-5-methylthiophene		0.061	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.071	2-Ethylthiazole		0.028	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.072	2-Ethylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.074	5-Ethylthiophene-2-carbaldehyde		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.076	2-Hexylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.078	2-Isobutyl-4,5-dimethylthiazole		0.12	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.080	2-Isopropyl-4,5-dimethylthiazole		0.012	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.084	5-Methyl-2-pentylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

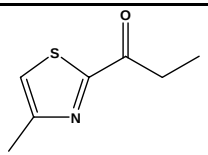
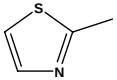
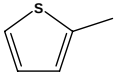
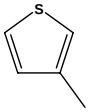
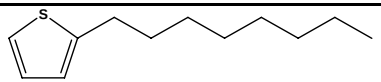
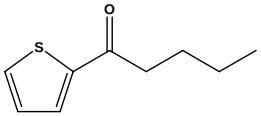
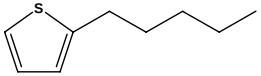
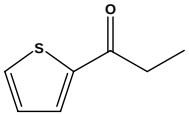
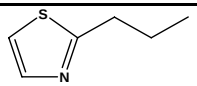
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.085	4-Methyl-2-propionylthiazole		0.0037	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.089	2-Methylthiazole		0.018	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.091	2-Methylthiophene		0.019	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.092	3-Methylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.093	2-Octylthiophene		0.012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.094	2-Pentanoylthiophene		0.0012	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.096	sec-Pentylthiophene		0.24	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.097	2-Propionylthiophene		0.12	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.098	2-Propylthiazole		0.085	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

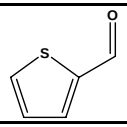
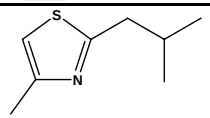
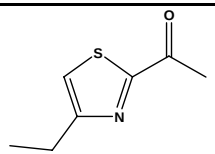
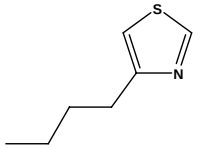
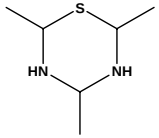
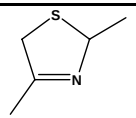
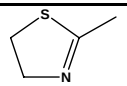
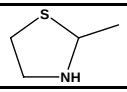
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.107	Thiophene-2-carbaldehyde		0.21	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.115	2-Isobutyl-4-methyl thiazole		0.011	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.116	2-Acetyl-4-ethylthiazole		0.024	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.118	4-Butylthiazole		1.3	Class II B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine		0.61	Class II B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.060	2,4-Dimethyl-3-thiazoline		0.012	Class II No evaluation			b)
15.086	2-Methyl-2-thiazoline		0.24	Class II No evaluation			b)
15.090	2-Methylthiazolidine		0.024	Class II No evaluation			c)

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

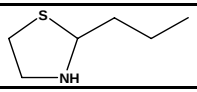
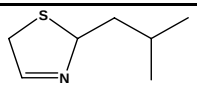
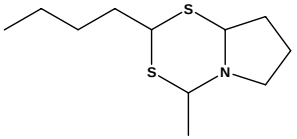
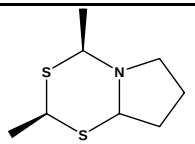
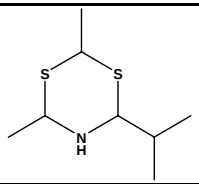
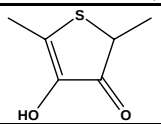
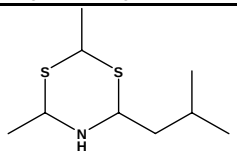
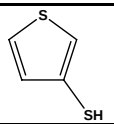
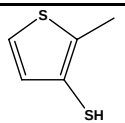
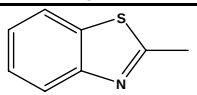
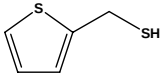
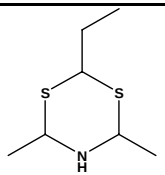
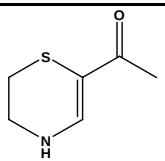
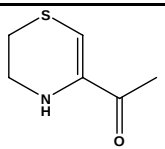
FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.099	2-Propylthiazolidine		0.012	Class II No evaluation			c)
15.119	2-Isobutyl-3-thiazoline		0.011	Class II No evaluation			b)
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine		0.0012	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine		0.055	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		1.5	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		5.7	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.082	3-Mercaptothiophene		0.011	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	

Table 2: Summary of Safety Evaluation Applying the Procedure (based on intakes calculated by the MSDI approach)

FL-no	EU Register name	Structural formula	MSDI 1) (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5]	Outcome on the material of commerce [6), 7), or 8)]	Evaluation remarks
15.087	2-Methyl-3-mercaptothiophene		0.12	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.088	2-Methyl-4,5-benzothiazole		0.0085	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		a)
15.106	Thiophene		0.12	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.108	2-Thiophenemethanethiol		0.0073	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	6)	
15.135	Ethyl thialdine		0.61	Class III B3: Intake below threshold, B4: No adequate NOAEL	Additional data required		
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine		0.012	Class III No evaluation			c)
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine		0.61	Class III No evaluation			c)

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
4) No safety concern based on intake calculated by the MSDI approach of the named compound.
5) Data must be available on the substance or closely related substances to perform a safety evaluation.
6) No safety concern at estimated level of intake of the material of commerce meeting the specification of Table 1 (based on intake calculated by the MSDI approach).
7) Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.

8) No conclusion can be drawn due to lack of information on the purity of the material of commerce.
a) Substance not supported by Industry (EFFA, 2009c).
b) Genotoxic potential *in vitro*.
c) Genotoxic potential *in vitro*. Substance not supported by Industry (EFFA, 2009c).

TABLE 3: SUPPORTING SUBSTANCES SUMMARY

Table 3: Supporting Substances Summary

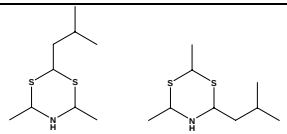
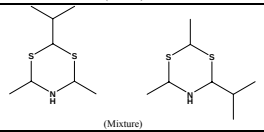
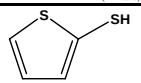
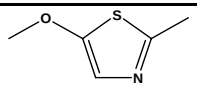
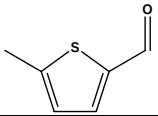
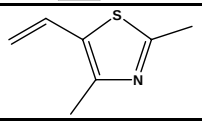
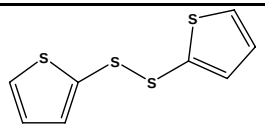
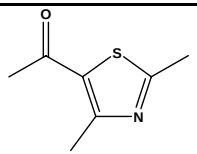
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
	2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3781 101517-87-7 and 101517-86-6	1046 JECFA specification (JECFA, 2002d)	0.1	No safety concern a)	Not in EU-Register.
	2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture)	 (Mixture)	3782 104691-41-0 and 104691-40-9	1047 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	Not in EU-Register.
15.001	2-Mercaptothiophene		3062 478 7774-74-5	1052 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Deleted b)	JECFA evaluated 2-thienyl mercaptan (CASrn as in Register).
15.002	2-Methyl-5-methoxythiazole		3192 736 38205-64-0	1057 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category B b)	
15.004	5-Methyl-2-thiophenecarbaldehyde		3209 2203 13679-70-4	1050 JECFA specification (JECFA, 2002d)	0.73	No safety concern a) Deleted b)	
15.005	2,4-Dimethyl-5-vinylthiazole		3145 2237 65505-18-2	1039 JECFA specification (JECFA, 2002d)	ND	No safety concern a) Category B b)	
15.008	2-Thienyl disulfide		3323 2333 6911-51-9	1053 JECFA specification (JECFA, 2002d)	0.061	No safety concern a) Category B b)	
15.011	5-Acetyl-2,4-dimethylthiazole		3267 2336 38205-60-6	1055 JECFA specification (JECFA, 2002d)	0.012	No safety concern a) Category B b)	JECFA evaluated 2,4-dimethyl-5-acetylthiazole (CASrn as in Register).

Table 3: Supporting Substances Summary

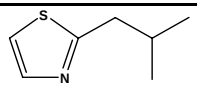
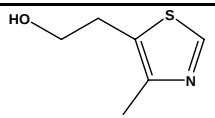
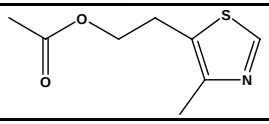
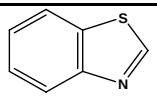
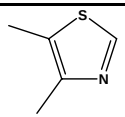
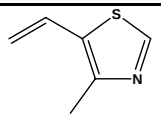
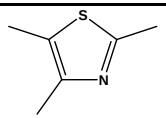
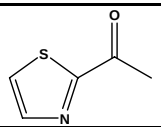
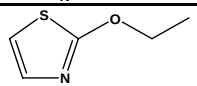
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
15.013	2-Isobutylthiazole		3134 11618 18640-74-9	1034 JECFA specification (JECFA, 2002d)	2.3	No safety concern a)	
15.014	5-(2-Hydroxyethyl)-4-methylthiazole		3204 11621 137-00-8	1031 JECFA specification (JECFA, 2002d)	150	No safety concern a)	
15.015	4-Methyl-5-(2-acetoxyethyl)thiazole		3205 11620 656-53-1	1054 JECFA specification (JECFA, 2002d)	8.6	No safety concern a)	JECFA evaluated 4-methyl-5-thiazoleethanol acetate (CASrn as in Register).
15.016	Benzothiazole		3256 11594 95-16-9	1040 JECFA specification (JECFA, 2002d)	1.2	No safety concern a)	
15.017	4,5-Dimethylthiazole		3274 11606 3581-91-7	1035 JECFA specification (JECFA, 2002d)	0.18	No safety concern a)	
15.018	4-Methyl-5-vinylthiazole		3313 11633 1759-28-0	1038 JECFA specification (JECFA, 2002d)	2.1	No safety concern a)	
15.019	2,4,5-Trimethylthiazole		3325 11650 13623-11-5	1036 JECFA specification (JECFA, 2002d)	0.61	No safety concern a)	
15.020	2-Acetylthiazole		3328 11726 24295-03-2	1041 JECFA specification (JECFA, 2002d)	9.7	No safety concern a)	
15.021	2-Ethoxythiazole		3340 11611 15679-19-3	1056 JECFA specification (JECFA, 2002d)	0.012	No safety concern a)	

Table 3: Supporting Substances Summary

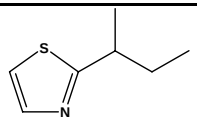
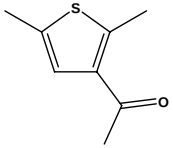
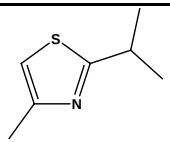
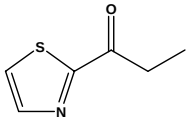
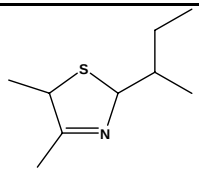
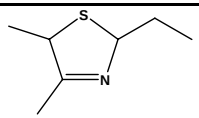
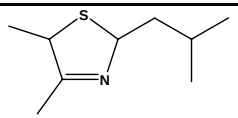
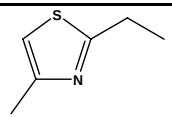
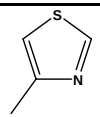
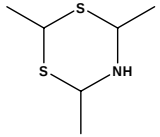
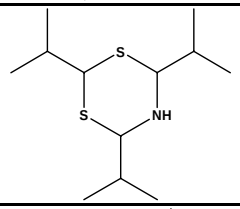
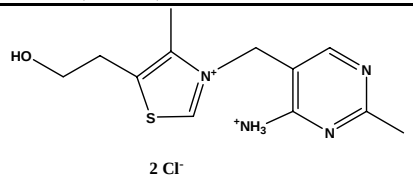
FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (μg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
15.022	2-(sec-Butyl)thiazole		3372 11598 18277-27-5	1033 JECFA specification (JECFA, 2002d)	0.024	No safety concern a)	JECFA evaluated 2-(1-methylpropyl)thiazole (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.
15.024	3-Acetyl-2,5-dimethylthiophene		3527 11603 2530-10-1	1051 JECFA specification (JECFA, 2002d)	18	No safety concern a)	
15.026	2-Isopropyl-4-methylthiazole		3555 15679-13-7	1037 JECFA specification (JECFA, 2002d)	19	No safety concern a)	
15.027	2-Propionylthiazole		3611 43039-98-1	1042 JECFA specification (JECFA, 2002d)	0.056	No safety concern a)	
15.029	2-(sec-Butyl)-4,5-dimethyl-3-thiazoline		3619 65894-82-8	1059 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 2-(2-Butyl)-4,5-dimethyl-3-thiazoline (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
15.030	4,5-Dimethyl-2-ethyl-3-thiazoline		3620 76788-46-0	1058 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 4,5-dimethyl-2-ethyl-3-thiazoline (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
15.032	4,5-Dimethyl-2-isobutyl-3-thiazoline		3621 65894-83-9	1045 JECFA specification (JECFA, 2002d)	0.012	No safety concern a)	JECFA evaluated 4,5-dimethyl-2-isobutyl-3-thiazoline (CASrn as in Register). (R)- or (S)-enantiomer not specified by CASrn in Register.

Table 3: Supporting Substances Summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) 1) (µg/capita/day)	SCF status 2) JECFA status 3) CoE status 4)	Comments
15.033	2-Ethyl 4-methylthiazole		3680 11612 15679-12-6	1044 JECFA specification (JECFA, 2002d)	3.2	No safety concern a)	
15.035	4-Methylthiazole		3716 11627 693-95-8	1043 JECFA specification (JECFA, 2002d)	0.097	No safety concern a)	
15.109	2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine		4018 11649 638-17-5	1049 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 2,4,6-trimethyldihydro-4H-1,3,5-dithiazine (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
15.113	5,6-Dihydro-2,4,6,tris(2-methylpropyl)4H-1,3,5-dithiazine		4017 74595-94-1	1048 JECFA specification (JECFA, 2002d)	ND	No safety concern a)	JECFA evaluated 2,4,6-triisobutyl-5,6-dihydro-4H-1,3,5-dithiazine (CASrn as in Register). (R)- or (S)- enantiomer not specified by CASrn in Register.
16.027	Thiamine hydrochloride		3322 10493 67-03-8	1030 JECFA specification (JECFA, 2002d)	300	No safety concern a)	

1) EU MSDI: Amount added to food as flavouring substance in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
2) Category 1: Considered safe in use, Category 2: Temporarily considered safe in use, Category 3: Insufficient data to provide assurance of safety in use, Category 4: Not acceptable due to evidence of toxicity.
3) No safety concern at estimated levels of intake.
4) Category A: Flavouring substance, which may be used in foodstuffs; Category B: Flavouring substance which can be used provisionally in foodstuffs.
a) (JECFA, 2002c).
b) (CoE, 1992).
ND) No intake data reported.

ANNEX I: PROCEDURE FOR THE SAFETY EVALUATION

The approach for a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), named the "Procedure", is shown in schematic form in Figure I.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999a), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure-activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes of 1800, 540 or 90 microgram/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996a).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- can the flavourings be predicted to be metabolised to innocuous products[8] (Step 2)?
- do their exposures exceed the threshold of concern for the structural class (Step A3 and B3)?
- are the flavourings or their metabolites endogenous[9] (Step A4)?
- does a NOAEL exist on the flavourings or on structurally related substances (Step A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to assure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warranted such actions.

[8] "Innocuous metabolic products": Products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997a).

[9] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997a).

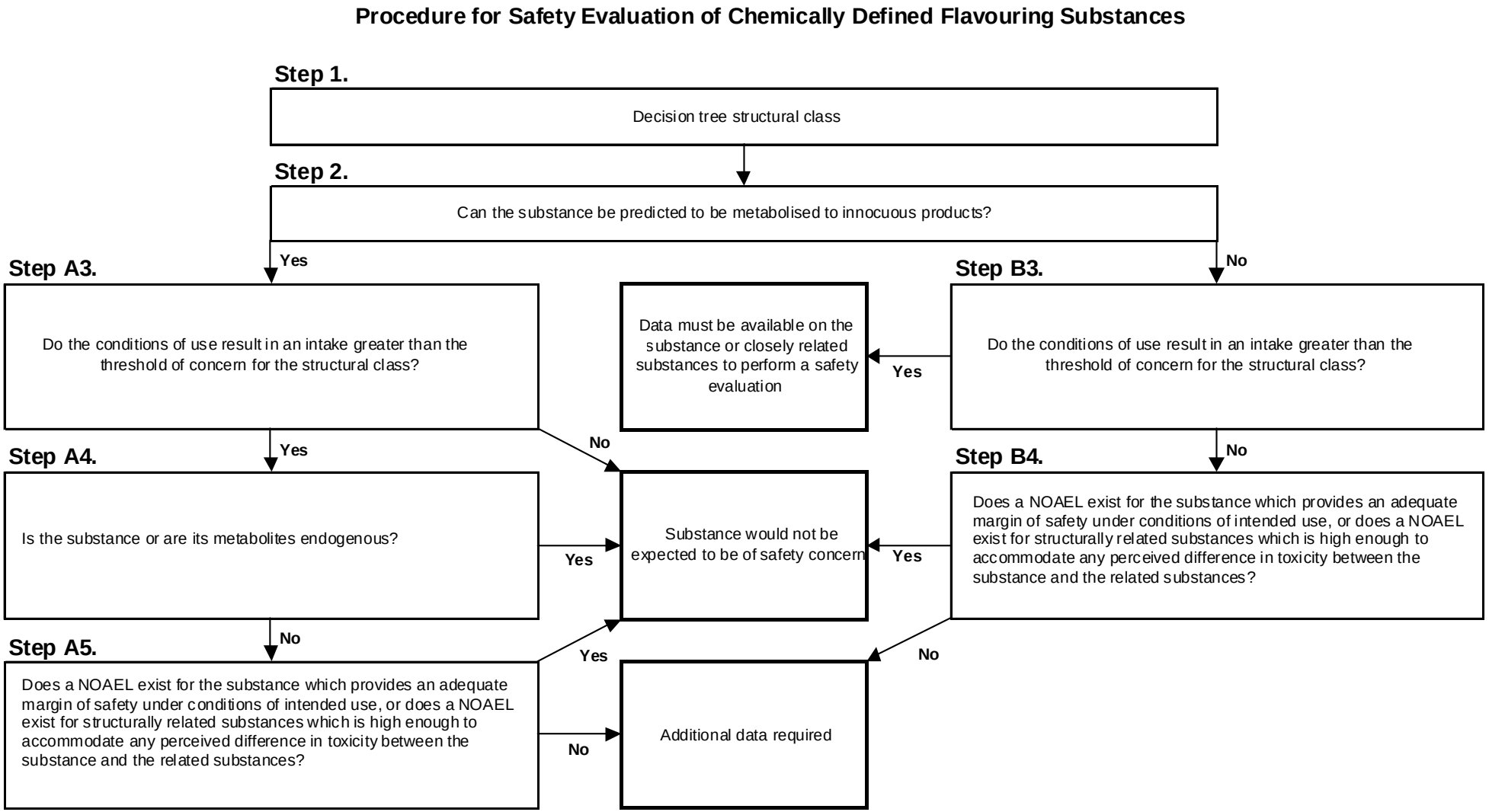


Figure I.1 *Procedure for Safety Evaluation of Chemically Defined Flavouring Substances*

ANNEX II: USE LEVELS / MTAMDI

II.1 Normal and Maximum Use Levels

For each of the 18 Food categories (Table II.1.1) in which the candidate substances are used, Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000a; Flavour Industry, 2004-5). According to the Industry the "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002i). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2002i).

Table II.1.1 Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000a)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds
05.0	Confectionery
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excl. dairy products
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0

The "normal and maximum use levels" are provided by Industry for 57 candidate substances in the present flavouring group (Table II.1.2).

Table II.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.21Rev3 (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5; Flavour Industry, 2010j).

FL-no	Food Categories Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
15.037	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.038	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.039	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	- -	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.040	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.042	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.043	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.044	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.045	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.050	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2

Table II.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.21Rev3 (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5; Flavour Industry, 2010j).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.051	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.052	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.054	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.055	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	0,4	2	0,4	0,4	-	-	1	2	1	2	5	1
15.058	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,1	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	0,5	2	5	1
15.060	0,4	0,2	0,2	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	1	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.061	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.062	0,4	0,2	0,4	0,3	0,4	-	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	-	1	0,2
	2	1	2	1,5	2	-	1	2	0,4	0,4	-	-	1	2	1	-	5	1
15.063	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.064	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.067	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.068	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	1	0,2	0,4	-	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	5	1	2	-	1
15.069	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.070	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.071	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.072	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.074	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.076	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.077	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.078	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.080	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.082	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.084	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.085	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.086	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.087	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.088	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.089	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	-	0,4	0,2	0,2	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	-	2	1	1	5	1
15.090	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1
15.091	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.092	0,2	0,1	0,2	0,2	-	0,2	0,1	0,2	0,1	0,1	-	-	0,1	0,2	0,1	0,2	0,4	0,1
	1	0,5	1	1	-	1	0,5	1	0,2	0,2	-	-	0,5	1	0,5	1	2	0,5
15.093	0,4	0,2	0,4	0,3	-	0,4	0,2	0,4	0,1	0,1	-	-	0,2	0,4	0,2	0,4	1	0,2
	2	1	2	1,5	-	2	1	2	0,4	0,4	-	-	1	2	1	2	5	1

Table II.1.2. Normal and Maximum use levels (mg/kg) for the candidate substances in FGE.21Rev3 (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5; Flavour Industry, 2010j).

FL-no	Food Categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
15.094	0,4 2	0,2 1	- -	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.096	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.097	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.098	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.099	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.106	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.107	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,1 0,5
15.108	0,2 1	0,1 0,5	0,2 1	0,2 1	- -	0,2 1	0,1 0,5	0,2 1	0,1 0,2	0,1 0,2	- -	- -	0,1 0,5	0,2 1	0,1 0,5	0,2 1	0,4 2	0,2 1
15.114	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.115	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.116	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.118	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.119	0,4 2	0,2 1	0,4 2	0,3 1,5	- -	0,4 2	0,2 1	0,4 2	0,1 0,4	0,1 0,4	- -	- -	0,2 1	0,4 2	0,2 1	0,4 2	1 5	0,2 1
15.129	0,1 2,5	0,1 2,5	1 25	0,1 2,5	0,1 2,5	10 250	2 50	1 25	0,1 2,5	0,1 2,5	- -	- -	0,1 2,5	- -	10 250	10 250	0,1 2,5	0,1 2,5
15.133	0,1 0,5	0,1 0,5	1 5	0,1 0,5	0,1 0,5	10 50	2 10	1 5	0,1 0,5	0,1 0,5	- -	- -	0,1 0,5	- -	10 50	10 50	0,1 0,5	0,1 0,5
15.135	1,5 3	1 2	- -	0,5 1	0,5 1	- -	0,5 1	1,5 3	1,5 3	- -	- -	- -	2 12	- -	- -	- -	0,5 1	- -

II.2 mTAMDI Calculations

The method for calculation of modified Theoretical Added Maximum Daily Intake (mTAMDI) values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table II.2.1. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table II.2.1 Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: Candy, confectionery	27.0
Exception b: Condiments, seasonings	20.0
Exception c: Alcoholic beverages	20.0
Exception d: Soups, savouries	20.0
Exception e: Others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in

Commission Regulation (EC) No 1565/2000 (EC, 2000a) and reported by the Flavour Industry in the following way (see Table II.2.2):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000a)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13, and/or 16 (EC, 2000a)
- Exception a (SCF, 1995) corresponds to food category 5 and 11 (EC, 2000a)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000a)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000a)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000a)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table II.2.2 Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000a) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation (EC) No1565/2000	**Distribution of the seven SCF food categories**		
Key	Food category	Food	Beverages	Exceptions
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (incl. mushrooms & fungi, roots & tubers, pulses and legumes), and nuts & seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, incl. flours & starches from roots & tubers, pulses & legumes, excluding bakery	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excl. dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat) - foods that could not be placed in categories 01.0 - 15.0	Food		

The mTAMDI values (see Table II.2.3) are presented for 57 candidate substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2004g; EFFA, 2004i; EFFA, 2007a; Flavour Industry, 2004-5; Flavour Industry, 2010j). The mTAMDI values are only given for the highest reported normal use levels (see Table II.2.3).

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (µg/person/day)**	**Structural class**	**Threshold of concern (µg/person/day)**
15.037	2-Acetyl-3-methylthiophene	78	Class II	540
15.038	2-Acetyl-4-methylthiazole	160	Class II	540
15.039	2-Acetyl-5-methylthiazole	160	Class II	540
15.040	2-Acetylthiophene	78	Class II	540

TableII.2.3 Estimated intakes based on the mTAMDI approach

FL-no	EU Register name	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
15.043	2-Butyl-5-ethylthiophene	78	Class II	540
15.044	2-Butylthiazole	160	Class II	540
15.045	2-Butylthiophene	78	Class II	540
15.050	2,5-Diethyl-4-methylthiazole	160	Class II	540
15.051	2,5-Diethyl-4-propylthiazole	160	Class II	540
15.052	2,5-Diethylthiazole	160	Class II	540
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	160	Class II	540
15.058	4,5-Dimethyl-2-ethylthiazole	130	Class II	540
15.061	2,5-Dimethyl-4-ethylthiazole	160	Class II	540
15.062	2,4-Dimethylthiazole	140	Class II	540
15.063	2,5-Dimethylthiazole	160	Class II	540
15.064	2,5-Dimethylthiophene	78	Class II	540
15.067	4-Ethyl-2-methylthiazole	160	Class II	540
15.068	5-Ethyl-2-methylthiazole	220	Class II	540
15.069	4-Ethyl-5-methylthiazole	160	Class II	540
15.070	2-Ethyl-5-methylthiophene	78	Class II	540
15.071	2-Ethylthiazole	160	Class II	540
15.072	2-Ethylthiophene	78	Class II	540
15.074	5-Ethylthiophene-2-carbaldehyde	78	Class II	540
15.076	2-Hexylthiophene	78	Class II	540
15.078	2-Isobutyl-4,5-dimethylthiazole	160	Class II	540
15.080	2-Isopropyl-4,5-dimethylthiazole	160	Class II	540
15.084	5-Methyl-2-pentylthiazole	160	Class II	540
15.085	4-Methyl-2-propionylthiazole	160	Class II	540
15.089	2-Methylthiazole	150	Class II	540
15.091	2-Methylthiophene	78	Class II	540
15.092	3-Methylthiophene	78	Class II	540
15.093	2-Octylthiophene	160	Class II	540
15.094	2-Pentanoylthiophene	160	Class II	540
15.096	sec-Pentylthiophene	160	Class II	540
15.097	2-Propionylthiophene	160	Class II	540
15.098	2-Propylthiazole	160	Class II	540
15.107	Thiophene-2-carbaldehyde	78	Class II	540
15.115	2-Isobutyl-4-methyl thiazole	160	Class II	540
15.116	2-Acetyl-4-ethylthiazole	160	Class II	540
15.118	4-Butylthiazole	160	Class II	540
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	4000	Class II	540
15.060	2,4-Dimethyl-3-thiazoline	160	Class II	540
15.086	2-Methyl-2-thiazoline	160	Class II	540
15.090	2-Methylthiazolidine	160	Class II	540
15.099	2-Propylthiazolidine	160	Class II	540
15.119	2-Isobutyl-3-thiazoline	160	Class II	540
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	160	Class III	90
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	160	Class III	90
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine		Class III	90
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	78	Class III	90
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine		Class III	90
15.082	3-Mercaptothiophene	78	Class III	90
15.087	2-Methyl-3-mercaptothiophene	78	Class III	90
15.088	2-Methyl-4,5-benzothiazole	160	Class III	90
15.106	Thiophene	78	Class III	90
15.108	2-Thiophenemethanethiol	78	Class III	90
15.135	Ethyl thialdine	250	Class III	90
15.114	5-Acetyl-2,3-dihydro-1,4-thiazine	160	Class III	90
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	4000	Class III	90

ANNEX III: METABOLISM

III.1. Introduction

The candidate substances in this FGE are structurally related to the 29 flavouring substances evaluated in "Safety Evaluations of Groups of Related Flavouring Agents: Sulfur-Containing Heterocyclic Compounds" (JECFA, 2002c).

The candidate substances from EU chemical groups 29 and 30 in this FGE include five- and six-membered sulphur-containing aromatic and non-aromatic heterocycles, which have been arranged into 9 subgroups in order to facilitate comparisons of the data sets between the groups. This division was done on the basis of degree of aromaticity and according to the presence of other heteroatoms (i.e. nitrogen). Subgroup A-I has been further divided into three subgroups: A-Ia (thiophene), A-Ib (thiophenes with alkyl, acyl or carbaldehyde ring substituents) and A-Ic (thiophenes with thiol-containing ring substituents). So, in total 11 subgroups.

The substances and subgroups are presented in Table III.1:

Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

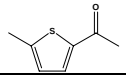
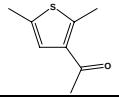
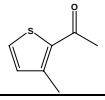
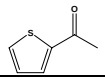
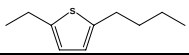
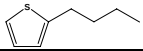
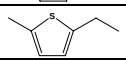
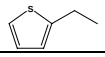
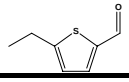
FL-no	EU Register name	Structural formula
A-Ia: Thiophene:		
15.106	Thiophene	
A-Ib: Thiophenes (with non-thiol-containing ring substituents):		
(15.004)	(5-Methyl-2-thiophenecarbaldehyde)	
(15.024)	(3-Acetyl-2,5-dimethylthiophene)	
15.037	2-Acetyl-3-methylthiophene	
15.040	2-Acetylthiophene	
15.043	2-Butyl-5-ethylthiophene	
15.045	2-Butylthiophene	
15.064	2,5-Dimethylthiophene	
15.070	2-Ethyl-5-methylthiophene	
15.072	2-Ethylthiophene	
15.074	5-Ethylthiophene-2-carbaldehyde	

Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

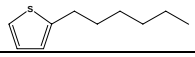
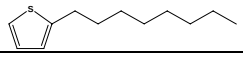
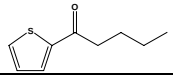
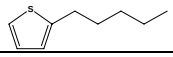
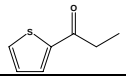
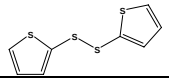
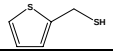
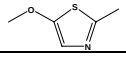
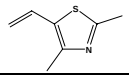
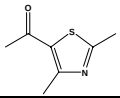
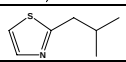
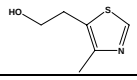
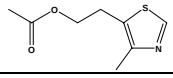
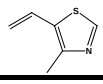
FL-no	EU Register name	Structural formula
15.076	2-Hexylthiophene	
15.091	2-Methylthiophene	
15.092	3-Methylthiophene	
15.093	2-Octylthiophene	
15.094	2-Pentanoylthiophene	
15.096	2-Pentylthiophene (Register name: sec-Pentylthiophene)	
15.097	2-Propionylthiophene	
15.107	Thiophene-2-carbaldehyde	
A-Ic: Thiophenes (with thiol-containing ring substituents):		
(15.001)	(2-Mercaptothiophene)	
(15.008)	(2-Thienyl disulfide)	
15.082	3-Mercaptothiophene	
15.087	2-Methyl-3-mercaptothiophene	
15.108	2-Thiophenemethanethiol	
A-II: Thiazoles:		
(15.002)	(2-Methyl-5-methoxythiazole)	
(15.005)	(2,4-Dimethyl-5-vinylthiazole)	
(15.011)	(5-Acetyl-2,4-dimethylthiazole)	
(15.013)	(2-Isobutylthiazole)	
(15.014)	(5-(2-Hydroxyethyl)-4-methylthiazole)	
(15.015)	(4-Methyl-5-(2-acetoxyethyl)thiazole)	
(15.017)	(4,5-Dimethylthiazole)	
(15.018)	(4-Methyl-5-vinylthiazole)	

Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

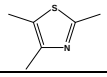
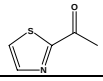
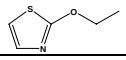
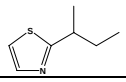
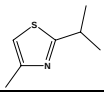
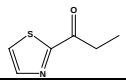
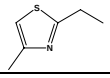
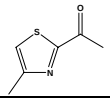
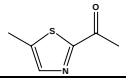
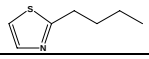
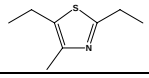
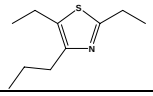
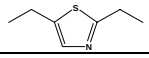
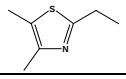
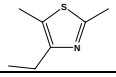
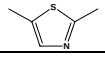
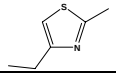
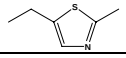
FL-no	EU Register name	Structural formula
(15.019)	(2,4,5-Trimethylthiazole)	
(15.020)	(2-Acetylthiazole)	
(15.021)	(2-Ethoxythiazole)	
(15.022)	(2-(sec-Butyl)thiazole)	
(15.026)	(2-Isopropyl-4-methylthiazole)	
(15.027)	(2-Propionylthiazole)	
(15.033)	(2-Ethyl 4-methylthiazole)	
(15.035)	(4-Methylthiazole)	
15.038	2-Acetyl-4-methylthiazole	
15.039	2-Acetyl-5-methylthiazole	
15.044	2-Butylthiazole	
15.050	2,5-Diethyl-4-methylthiazole	
15.051	2,5-Diethyl-4-propylthiazole	
15.052	2,5-Diethylthiazole	
15.058	4,5-Dimethyl-2-ethylthiazole	
15.061	2,5-Dimethyl-4-ethylthiazole	
15.062	2,4-Dimethylthiazole	
15.063	2,5-Dimethylthiazole	
15.067	4-Ethyl-2-methylthiazole	
15.068	5-Ethyl-2-methylthiazole	
15.069	4-Ethyl-5-methylthiazole	

Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

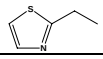
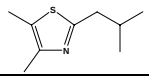
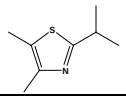
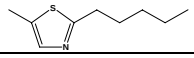
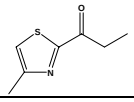
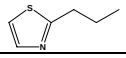
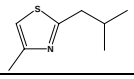
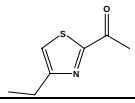
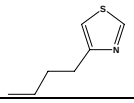
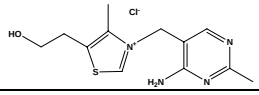
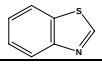
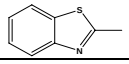
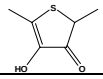
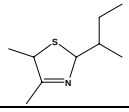
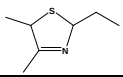
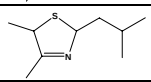
FL-no	EU Register name	Structural formula
15.071	2-Ethylthiazole	
15.078	2-Isobutyl-4,5-dimethylthiazole	
15.080	2-Isopropyl-4,5-dimethylthiazole	
15.084	5-Methyl-2-pentylthiazole	
15.085	4-Methyl-2-propionylthiazole	
15.089	2-Methylthiazole	
15.098	2-Propylthiazole	
15.115	2-Isobutyl-4-methylthiazole	
15.116	2-Acetyl-4-ethylthiazole	
15.118	4-Butylthiazole	
(16.027)	(Thiamine hydrochloride)	
A-III: Benzothiazoles:		
(15.016)	(Benzothiazole)	
15.088	2-Methyl-4,5-benzothiazole	
B-I : Dihydrothiophenes:		
15.077	4-Hydroxy-2,5-dimethylthiophen-3(2H)-one	
B-II: Thiazolines:		
(15.029)	(2-(sec-Butyl)-4,5-dimethyl-3-thiazoline)	
(15.030)	(4,5-Dimethyl-2-ethyl-3-thiazoline)	
(15.032)	(4,5-Dimethyl-2-isobutyl-3-thiazoline)	
15.060	2,4-Dimethyl-3-thiazoline	

Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

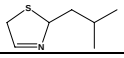
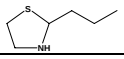
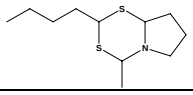
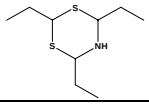
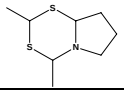
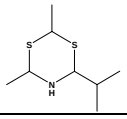
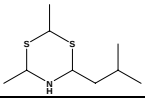
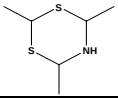
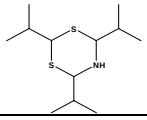
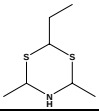
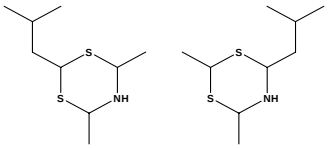
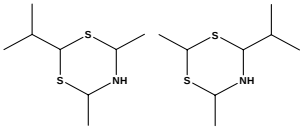
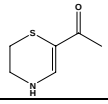
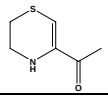
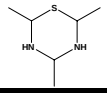
FL-no	EU Register name	Structural formula
15.086	2-Methyl-2-thiazoline	
15.119	2-Isobutyl-3-thiazoline	
B-III: Thiazolidines:		
15.090	2-Methylthiazolidine	
15.099	2-Propylthiazolidine	
B-IV: Dithiazines:		
15.042	2-Butyl-4-methyl(4H)pyrrolidino[1,2d]-1,3,5-dithiazine	
15.054	Dihydro-2,4,6-triethyl-1,3,5(4H)-dithiazine	
15.055	2,4-Dimethyl(4H)pyrrolidino[1,2e]-1,3,5-dithiazine	
15.057	4,6-Dimethyl-2-(1-methylethyl)dihydro-1,3,5-dithiazine	
15.079	2-Isobutyldihydro-4,6-dimethyl-1,3,5-dithiazine	
(15.109)	(2,4,6-Trimethyldihydro-1,3,5(4H)-dithiazine)	
(15.113)	(5,6-Dihydro-2,4,6,tris(2-methylpropyl)4H-1,3,5-dithiazine)	
15.135	Ethyl thialdine	
(Not in EU Register)	(2-Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	 (Mixture)
(Not in EU Register)	(2-Isopropyl-4,6-dimethyl 2,6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	 (Mixture)
B-V: Dihydrothiazines:		

Table III.1 Division of candidate and supporting substances into structurally related subgroups – supporting substances are listed in brackets

FL-no	EU Register name	Structural formula
15.114	6-Acetyl-2,3-dihydro-1,4-thiazine (Register name: 5-acetyl-2,3-dihydro-1,4-thiazine)	
15.133	5-Acetyl-2,3-dihydro-1,4-thiazine	
B-VI: Thiadiazines:		
15.129	Tetrahydro-2,4,6-trimethyl-1,3,5(2H)-thiadiazine	

The following is a description of the characteristic features of the 9 subgroups:

A: Aromatic group:

Subgroup A-I: Thiophenes (20 candidate substances and four supporting substances): aromatic heterocyclic five-membered ring substances with a sulphur atom at ring position 1. This group is further divided into three subgroups: A-Ia with thiophene, A-Ib with 16 ring-substituted thiophenes which have one or more alkyl, acyl or carbaldehyde substituents to the ring and subgroup A-Ic with three ring-substituted thiophenes which have a free thiol group in their ring-substituent group.

Subgroup A-II: Thiazoles (23 candidate substances and 17 supporting substances): five-membered *aromatic* heterocycles containing one sulphur and one nitrogen atom in the 1- and 3-ring positions, respectively. To this heterocyclic ring one or more alkyl- or acyl- substituents may be attached.

Subgroup A-III: Benzothiazoles (one candidate substance and one supporting substance): a bicyclic molecule consisting of a five-membered *aromatic* heterocycle, containing one sulphur and one nitrogen atom in the 1- and 3-ring positions, respectively, with a fused benzene ring at the 4- and 5-ring positions.

B: Non-aromatic group:

Subgroup B-I: Dihydrothiophenes (one candidate substance and no supporting substances): heterocyclic five-membered ring substance with a sulphur atom at ring position 1. Only one double bond is present in the ring. The ring bears several substituents.

Subgroup B-II: Thiazolines (three candidate substances and three supporting substances): five-membered heterocycles containing a sulphur and a nitrogen atom in the 1- and 3-ring positions, respectively. In the ring, one double bond is present (in contrast to the thiazoles, which have two double bonds).

Subgroup B-III: Thiazolidines (two candidate substances and no supporting substances): five-membered heterocycles containing a sulphur and a nitrogen atom in the 1- and 3-ring positions, respectively. In the ring no double bonds are present (*cf.* thiazoles and thiazolines).

Subgroup B-IV: Dithiazines (six candidate substances and two supporting and two structurally related substances): six-membered non-aromatic heterocycles containing two sulphur atoms, and one nitrogen atom, with various ring substituents, including a fused second ring in two of these.

Subgroup B-V: Dihydrothiazines (two candidate substances and no supporting substances): a six-membered heterocyclic ring, containing a sulphur and a nitrogen atom in para positions, and one double bond. The ring bears an acetyl substituent group.

Subgroup B-VI: Thiadiazines (one candidate substance and no supporting substances): six-membered non-aromatic heterocycles containing one sulphur atoms, and two nitrogen atoms in para-positions, with three methyl substituent groups.

III.2. Absorption, Distribution and Elimination

Only limited data were submitted for the evaluation of the absorption, distribution and elimination of the candidate flavouring substances in this group. The only candidate substance for which absorption and elimination were studied is thiophene [FL-no: 15.106]. Data were also submitted for a few more or less related substances. Because the information is so fragmentary, it is not useful to present it (sub)group-wise. In addition, some of the studies are related to oral exposure and some others to parenteral dosing, and the relevance of the latter may be limited.

Candidate substances

Thiophene [FL-no: 15.106]

Single doses of 33 mg/kg bw [2,5-^{14}C]-thiophene were administered to mice via oral and rectal intubation. At 48 hours after the oral gavage dose, 78 % of the radioactivity was found in the urine, while 5 % was found in both faeces and expired air. At 24 hours after the rectal dose, 43 % was found in the urine, but 40 % was found in the expired air. The difference in the elimination pattern after oral *vs.* rectal administration was attributed to an extensive first-pass effect after the oral dose. Also, the oral dose resulted in 10 times higher levels of radioactivity in the liver as compared to the rectal dose. With thin-layer chromatography two urinary metabolites were observed. However, none of these metabolites and none of the radioactivity in tissues or excreta were further identified (Chanal et al., 1974).

Female rats (200 - 250 g) and rabbits (2 - 3 kg) were administered thiophene as single oral gavage doses of 60 mg and 450 mg, respectively. Urine was collected over a 24-hours period following dosing. Table III.2 provides a summary of the quantitative determinations of metabolites in this study.

Table III.2 Summary of excretion of metabolites of thiophene in rabbits and rats

Metabolites	Rabbit (N=6)	Rat (N=3)
(Pre)-mercapturic acids in urine	38 (32 – 49)	40 (36 – 45)
Free thiophene expired	Not determined	32 (29 – 35)
Free thiophene in faeces	Not determined	0.48 (0.25 – 0.68)

Tabulated data are percentage of administered dose (%)

Other urinary metabolites, in particular conjugates of 2-hydroxy- or 3-hydroxythiophene, any acid-hydrolysable products of thiophene or free thiophene were not found (Bray et al., 1971).

When male Sprague-Dawley rats were injected intraperitoneally with a 200 mg/kg bw dose of tritiated (at C2 and C5 positions) thiophene in corn oil, approximately 31 % of the radioactivity was excreted between 0 and 15 hours, while 4 % of the radioactivity was excreted between 15 and 50 hours. More than 94 % of the

urinary radioactivity was accounted for by a single metabolite, the 2-mercapturic acid derivative of 1,4-dihydrothiophene-*S*-oxide (Dansette et al., 1992).

In rats, a single subcutaneous dose of ^{35}S-thiophene was rapidly absorbed into the blood stream. Thiophene produced a peak blood concentration within 30 minutes and peak levels in the brain within three hours. Radioactivity was excreted in the urine at 61.1 % of the dose within 34 hours and 74.4 % of the dose within three days (Bikbulatov and Nigmatullina, 1976; O'Donoghue, 2000).

Non-candidate substances:

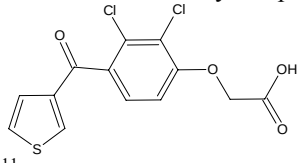
In two male Sprague-Dawley rats, approximately 20 % of the radioactivity of an intraperitoneal injection of 30 mg/kg bw of the 3-aroylthiophene (tienilic acid isomer 1)[10] (^{14}C at the keto position) is recovered in the urine within 24 hours. Approximately 15 % of urinary radioactivity could be accounted for as a mixture of two diastereoisomers of the mercapturic acid conjugate of the 3-aroyl-4,5-dihydrothiophene. The study was designed to identify these two metabolites. The rest of the urinary radioactivity was identified as parent substance (*ca.* 17 % of the dose) and an unidentified metabolite (*ca.* 1 % of the dose), presumably a derivative of one or both of the already identified mercapturates (Valadon et al., 1996).

When the benzothiazole derivative fostedil[11] was administered to dogs via intravenous infusion or orally as solution, suspension or as encapsulated substance, mean relative bioavailabilities for the parent substance after oral dosing could be calculated to be 71, 64 or 42 %, respectively. The substance was cleared from the plasma with a terminal half-life of 6 - 9 hours and based on high biliary excretion, enterohepatic circulation has been suggested. When the substance was administered with a radiolabel (no further details) 80 % of the radioactivity was found in the faeces (Thomas, 1984; Thomas and Bopp, 1984).

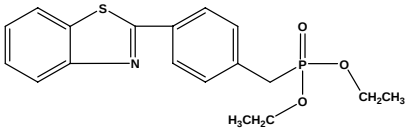
In a previous FGE.24Rev1 (EFSA, 2008t) data on the absorption and elimination of indole[12] have been discussed. As this substance bears some resemblance to the benzothiazoles in subgroup A-III, these data are also briefly mentioned here. After oral dosing of 2-^{14}C-indole to rats 62 - 82 % of the administered radioactivity was excreted via urine or expired air within 48 - 72 hours post-dosing.

The substances in subgroup B-IV (the dithiazines with FL-no: 15.042, 15.054, 15.055, 15.057, 15.079 and 15.135) may be regarded as cyclic thioacetals, which could be subject to acid hydrolysis in the stomach, similar to oxygen-containing acetals. However, thioacetals are more resistant to hydrolysis than oxygen acetals. In addition, chances of hydrolysis would be reduced even more by their cyclic character (Smith and

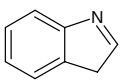
[10] Structure of 3-aroylthiophene (isomer of tienilic acid (2-aroylthiophene)):



[11] Structure of fostedil:



[12] Strucure of indole:



March, 2001; EFSA, 2011u). It is thus anticipated that these substances may be expected to reach the intestinal lumen intact and may also be absorbed as such.

III.3. Metabolism

In the sections below, the metabolism data available have been presented according to the division into subgroups of the candidate flavouring substances in this FGE.

III.3.1. Aromatic candidate substances

Subgroup A-Ia thiophene and A-Ib thiophenes with non-thiol-containing ring substituents

(Candidate substances [FL-no: 15.037, 15.040, 15.043, 15.045, 15.064, 15.070, 15.072, 15.074, 15.076, 15.091, 15.092, 15.093, 15.094, 15.096, 15.097, 15.106 and 15.107])

Candidate substances:

Thiophene [FL-no: 15.106]

Thiophene was administered to female rats (200 - 250 g) and rabbits (2 - 3 kg) as single oral gavage doses of 60 mg and 450 mg, respectively. Urine was collected over a 24-hours period following dosing. The predominant metabolites excreted in the urine were a mercapturic acid and a premercapturic acid, which according to the study authors could result from the conjugation of glutathione with an intermediate 2,3-thiophene-epoxide. 2-Thienylmercapturic acid was excreted in relatively small quantities and 3-hydroxy-2,3-dihydro-2-thienyl-mercapturic acid was the major metabolite excreted in urine. The metabolites were identified by IR spectroscopy, GLC and MS techniques and further by characteristic chemical reactions of thiophene derivatives (desulphuration following treatment of the premercapturic acid with Raney nickel resulting in the formation of 2-butanol) (Bray et al., 1971).

More recently, male Wistar rats (190 - 400 g) were injected intraperitoneally with 0.2 mmol (= ~ 17 mg) thiophene in peanut oil, and mercapturic acids were not found to be important thioethers (i.e. comprising not more than about 1 % of the dose) excreted in the urine (Hickman et al., 1992), contrary to the results of Bray *et al* (Bray et al., 1971). It is noted that 2-thienyl mercapturic acid was also identified as a less important metabolite by Bray et al. (1971) and that the two studies differ in route of administration. The oral route of administration (Bray et al., 1971) results in higher metabolic conversion as compared to the intraperitoneal one (Hickman et al., 1992). In addition, identification of thioethers by Hickman et al. (1992) was only done by reaction with Ellman's reagent following extraction and hydrolysis of the urine samples. No attempts were made to identify any other thiophene metabolite in urine samples.

When male Sprague-Dawley rats were injected (intraperitoneal) with 200 mg/kg bw [2,5-^{3}H]-labelled thiophene, more than 94 % of the urinary radioactivity was accounted for by the 2-mercapturic acid derivative of 2,5-dihydrothiophene-*S*-oxide, as identified by NMR, IR and Mass spectroscopical techniques. A small second peak in the HPLC diagram of the urine radioactivity was also observed, but this peak was not further studied. The authors concluded that its formation occurred by *S*-oxidation yielding the very reactive thiophene sulphoxide, which undergoes Michael-type addition of glutathione at the 2-position. In subsequent reactions, the glutathione moiety of this metabolite is converted into an N-acetyl cysteine group. The final metabolite is subsequently excreted via urine, and may be converted spontaneously into 2-thienyl-mercapturic acid (Dansette et al., 1992). The authors considered the formation of the epoxide intermediate, as suggested by Bray et al. (1971), highly unlikely.

Based on subsequent studies with chemically defined reaction mixtures and with microsomal incubations, the formation of the 2,5-dihydrothiophene-*S*-oxide was further substantiated by the formation of two diastereoisomeric thiophene *S*-oxide dimers (3a,4,7,7a-tetrahydro-4,7epithiobenzo[*b*]thiophene 1,8 dioxide) one of which (the 1-*syn,-8-syn,endo*-isomer) was suggested having been observed in the *in vivo* studies by Dansette et al (*ca.* 10 % of urinary radioactivity, comprising 2 % of the oral dose in the above study; the unidentified second peak (see above)). The formation of the dimers may result from a Diels−Alder reaction. Thus, the metabolic fate for the thiophene *S*-oxide *in vivo* involves predominantly reaction with nucleophiles such as glutathione (catalysed by glutathione S-transferase) leading eventually to mercapturate derivatives that are excreted in the urine, or to a lesser extent it involves dimerization and the resulting dimer is also excreted via the urine (Treiber et al., 1997).

Non-candidate substances:

In two male Sprague-Dawley rats injected i.p. with the 3-aroylthiophene tienilic acid isomer 1 (see above), 15 % of urinary radioactivity (~ 2 % of the dose) could be accounted for as a mixture of two diastereoisomers of the mercapturic acid conjugate of 3-aroyl-4,5-dihydrothiophene. In the ultimate urinary metabolite, the *N*-acetylcysteine residue (mercapturate) is found in the 4-position of the thiophene ring. This residue may be introduced at that position after initial Michael-type addition of glutathione to the primary thiophene sulphoxide metabolite (see Figure III.1) at the ring position 2 (i.e. the C next to the S atom) and several rearrangements, including ring opening / closing and reaction with second GSH molecules. Results of *in vitro* experiments with rat liver microsomes indicate that the thiophene sulphoxide is a reactive intermediate in the conversion of the 3-aroylthiophene to the dihydromercapturic acid metabolite (Valadon et al., 1996).

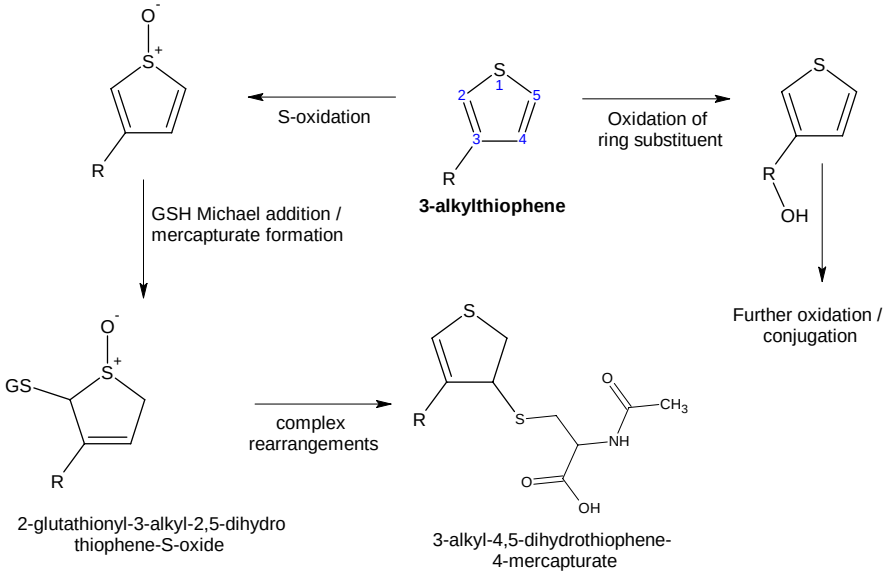


Figure III.1. *Proposed metabolism of 3-alkylthiophenes*

Oxidation of 2-phenylthiophene by rat liver microsomes, in the presence of NADPH and glutathione (GSH) involves two initial reactions, resulting in three subsequent types of metabolites. The first reaction involves *S*-oxidation to yield 2-phenylthiopene S-oxide that then dimerizes by Diels–Alder type reaction of the *S*-oxide. Alternatively, the S-oxide alternatively may form a glutathione adduct via a 1,4-Michaël-type addition of glutathione to the C5 position of 2-phenylthiopene S-oxide. The second metabolic reaction involves formation of a 2-phenylthiophene-4,5-epoxide which may be subject to subsequent conjugation with

glutathione at C4 of the thiophene ring. Subsequent dehydration of the resulting hydroxyl-glutathione conjugate yields the corresponding glutathione conjugate of phenylthiopene which is excreted mainly as the 2-mercapturic derivative. Oxidation of 2-phenylthiophene by recombinant, human cytochrome P450 1A1, in the presence of NADPH and glutathione, also led to these metabolites. These results provide the evidence that cytochrome P450 (1A1) may catalyze the oxidation of thiophene compounds with the simultaneous formation of two reactive intermediates, a thiophene-S-oxide and a thiophene epoxide (Dansette et al., 2005).

The addition of alkyl substituents can result in a significant change in metabolism. Biotransformation of a thiophene derivative was studied in six male healthy volunteers after administration of a single oral dose of 12.5 mg olanzapine[13]. Mean radiocarbon recovery was 87 %, with 30 % appearing in the feces and 57 % excreted in the urine. In addition to other metabolites, the methyl substituent on the thiophene ring underwent oxidation to the corresponding 2-hydroxymethyl and 2-carboxylic acid derivatives. There was no evidence for glutathione conjugation of the thiophene S-oxide or epoxidation of the thiophene ring double bond for alkyl-substituted thiophenes (Kassahun et al., 1997). However, the total recovery and identification of metabolites in urine or faeces was far from complete and some metabolites may have been missed in the analysis. In addition, reactions alternative to S-oxidation (e.g. N-oxidation or n-glucuronidation) may greatly reduce the relevance of the S-oxidation in this substance as compared to more simple alkyl-substituted thiophenes.

Subgroup A-Ic: Thiophenes with a thiol group in the ring substituent

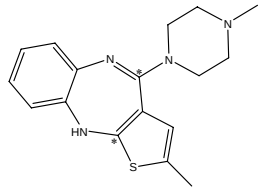
Candidate substances [FL-no: 15.082, 15.087 and 15.108]

There is no specific information on any of the candidate substances in this subgroup, or on stucturally related thiophene derivatives. It may be anticipated that some of the metabolic conversions described above for subgroups A-Ia and A-Ib may also apply to this subgroup.

In addition, the mercapto-group (thiol) in the ring-substituent chain may undergo *S*-methylation to produce the corresponding methyl thioether or sulphide, with further oxidation to the corresponding sulphoxide and sulphone. They may also react with glutathione or other endogenous thiol substances to form mixed disulphides, which may undergo reduction to thiols, or oxidative desulphuration. Alternatively, they may undergo enzymatic oxygenation resulting in the formation of the corresponding sulphinic or sulphonic acid. All these metabolites are expected to be excreted in the urine. A more detailed discussion on the metabolism of sulphur compounds may be found in an other EFSA-CEF Opinion on other sulphur-containing flavouring substances (EFSA, 2011h).

Additional considerations for the candidate substances in Subgroup A-I

No information was submitted that would indicate whether side chain oxidation of the alkyl-substituted thiophenes or keto-reduction of the acyl-substituted thiophenes could occur. Such reactions may be anticipated (e.g. omega or omega-1 oxidations). Similar reactions have been discussed for alkylated furane and pyrazines in previous Opinions (EFSA, 2010h; EFSA, 2011h). In addition, the two aldehyde candidate substances [FL-no: 15.074 and 15.107] may be expected to be oxidised to the corresponding carboxylic



13 Structure of Olanzapine; the radioactive carbons are indicated with *.

acids. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (EFSA, 2009f; EFSA, 2010h; EFSA, 2011h).

Rance (1989) has indicated that unsubstituted thiophene may be subject to S-oxidation and ring substitution. It was stated that S-oxides of thiophene are highly reactive. In this review several studies have been quoted with C2 substituted thiophene derivatives (in particular the pharmaceuticals tienilic acid, morantel and pyrantel) for which it was demonstrated that ring C5 hydroxylation of these substituted thiophenes may occur to a considerable extent. In contrast C2, C5 disubstituted thiophenes are less susceptible to ring hydroxylation (Rance, 1989).

Subgroup A-II: Thiazoles

Candidate substances [FL-no: 15.038, 15.039, 15.044, 15.050, 15.051, 15.052, 15.058, 15.061, 15.062, 15.063, 15.067, 15.068, 15.069, 15.071, 15.078, 15.080, 15.084, 15.085, 15.089, 15.098, 15.115, 15.116 and 15.118]

No specific information was available for any of the candidate substances in this subgroup.

Non-candidate substances:

Thiamine hydrochloride [FL-no: 16.027] and 5-(2-hydroxyethyl)-4-methylthiazole [FL-no: 15.014]

Thiamine (see Figure III.2) is excreted in the urine as unchanged substance but also in the form of 25 - 30 metabolites, some of which still contain the pyrimidine moiety, while other metabolites are generated as a result of molecular cleavage.

In fish and micro-organisms, breakdown of thiamine is catalysed by two enzymes, namely thiaminase I and thiaminase II (Tietz, 1986a; Tanphaichitr, 1976). Based on comparative studies in which rodents were given thiamine intravenously or orally, it has been demonstrated that in the gastrointestinal tract thiamine may already be split into a pyrimidine and a thiazole derivative, namely 5-(2-hydroxyethyl)-4-methylthiazole, which together with its oxidation product (4-methylthiazole-5-acetic acid) and parent thiamine have been identified in urine. The fate of the pyrimidine derivative will not be further discussed. The relevance of the intraintestinal cleavage may be different among various animal species (Informatics Inc., 1974).

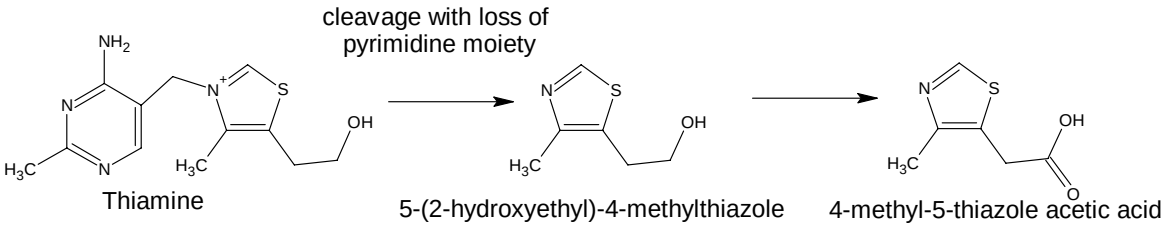


Figure III.2. *Metabolism of thiamine and 5-(2-hydroxyethyl)-4-methylthiazole*

Chlormethiazole

The therapeutic agent chlormethiazole (5-(2-chloroethyl)-4-methylthiazole; see Figure III.3), is an alkyl- and chloroalkyl-substituted thiazole derivative that is metabolised by C-oxidation of the alkyl- and chloroalkyl-substituents and by S- and N-oxidation. In male humans, a 1000 mg oral dose of chlormethiazole is metabolised via side-chain C-oxidation of the ethyl C1-position to yield 5-(1-hydroxyethyl)-4-methylthiazole, 5-acetyl-4-methylthiazole, 5-(1-hydroxy-2-chloroethyl)-4-methylthiazole, 4,5-dimethylthiazole and 4-methylthiazole-5-acetic acid, which is the major C-oxidation urinary metabolite (see Figure III.3). Presumably, the 5-methyl derivative is formed via decarboxylation of the 5-acetic acid

metabolite. Additionally, oxidation of the methyl group at C4 in 5-(2-hydroxyethyl)-4-methylthiazole yields 5-(2-hydroxyethyl)-4-thiazolecarboxylic acid lactone.

Separate ring sulphoxide and ring N-oxide products were not identified, but a combined ring S- and N-oxide of a side-chain oxidised metabolite was also eliminated in the 12-hour urine of volunteers. This was the first reported example of a sulphoxidation of a thiazole sulphur and simultaneous oxidation of two different heteroatoms in the same heteroaromatic ring *in vivo* (Offen et al., 1985).

Moore et al. (1975) reported the excretion of urinary metabolites of chlormethiazole after oral administration of this anticonvulsant drug (in the form of its unstable chlormethiazole ethanedisulphonate derivative) to humans. The substance was given in capsules to three volunteers (sex unknown) in an amount of 768 mg. Urinary samples were collected for up to 36 hours post-dosing and processed for the identification and quantification of metabolites by GS-MS techniques. Results of urinalysis were compared to results obtained with reference substances. After oral administration, about 16 % of the dose could be identified in the 36 hours urine as 4 substances, parent chlormethiaziole (< 0.01 % of the dose), 5-acetyl-4-methylthiazole, 5-(1-hydroxyethyl)-4-methylthiazole (free and as unspecified conjugate) and 4-methylthiazole-5-acetic acid (> 75 % of all metabolites identified). 5-(2-Hydroxyethyl)-4-methylthiazole was only found in trace amounts. In addition, a metabolite was tentatively identified as 4-methyl-5-thiazole-acetaldehyde. The authors noted that at that time 80 % of the dose was still unaccounted for, which might indicate extensive metabolism of the substance in humans (Moore et al., 1975).

It has also been reported that chlormethiazole undergoes nucleophilic attack at C2, which would ultimately result in ring opening. The exact mechanism for the formation of ring opening products has not been well-described/identified, but the products are nonetheless found in human urine. It was suggested that N-oxidation followed by glutathione conjugation and subsequent glutathione break-down would yield the 2-thiomethyl derivative of chlormethiazole, whereas hydroxide ion attack would yield 2,4-pentanedione-3-thiol and thiodiacetic acid. The authors suggest that the N-oxidation would make the C2 prone to nucleophilic attack (Grupe and Spiteller, 1982; Pal and Spiteller, 1982). According to data from Grupe & Spiteller (1982), urinary excretion of thiodiacetic acid may comprise up to about 5 % of the dose of chlormethiazole in humans. For the other metabolites (see Figure III.3) no quantitative data were provided.

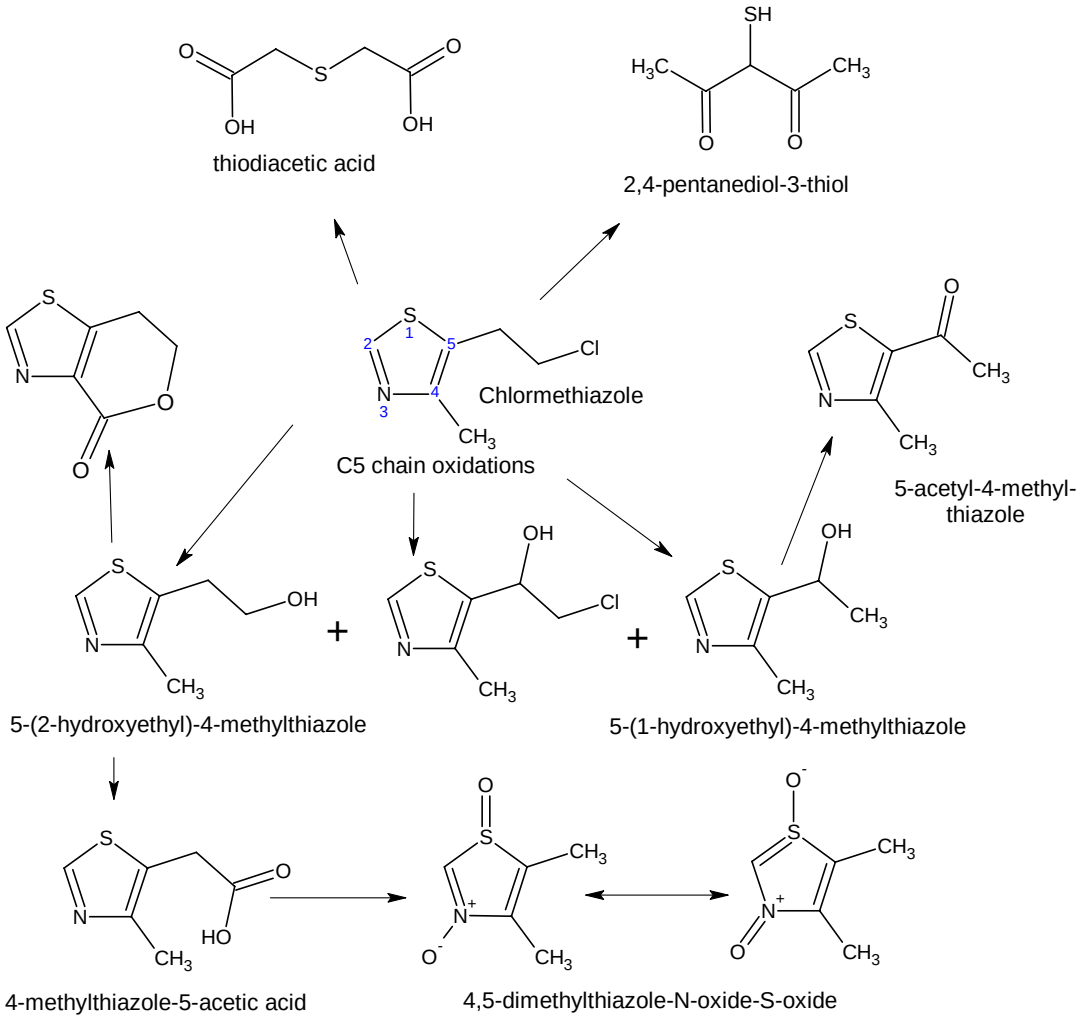


Figure III.3. *Metabolism of Chlormethiazole.*

Herbertz et al. (1973) studied the metabolism of (2-^{14}C)-chlormethiazole-ethane disulphonate in isolated recycling-perfused rat liver. After 2 hours, 35 % of the system's radioactivity was found in the bile, which activity was associated with a polar metabolite, not observed in previous *in vivo* studies in urine. This metabolite was identified as a mixture of N,4-dimethyl-thiazole-5-acetic acid, 2-hydroxy-N,4-dimethyl-thiazole-5-acetic acid and the glycine conjugate of the latter. Other substances in the perfusate were chlormethiazole, 2-hydroxy-4-methyl-5-(2-chloroethyl)thiazole, 4-methyl-5-(2-hydroxyethyl)thiazole, 4-methylthiazole-5-acetic acid, 2-hydroxy-4-methylthiazole-5-acetic acid and its glycine conjugate (Herbertz et al., 1973).

In vivo, the urine was identified as the primary route of elimination (70 % of the dose within 3 hours in a study by Allgen et al., 1963 (cited by Herbertz et al., 1973), with no formation of N-methylated products. Hence, it was speculated that *in vivo* enterohepatic recycling of chlormethiazole-metabolites would occur, in particular of the N-methylated substances, which would be de-methylated in the gastro-intestinal tract and subsequently be excreted via the kidneys. It was noted that the difference between the observations *in vivo* and the perfused system may have occurred because of the build-up of (secondary) metabolite levels in the (closed) perfused system (Herbertz et al., 1973).

Mizutani et al. (1993) investigated the relationship between the toxicological profile of several thiazole derivatives and the toxicity of the ring cleavage products in particular the thioamide metabolites (see Figure III.4). The various thiazoles were nephrotoxic *in vivo* in GSH-depleted mice, which toxicity decreased upon

substitution at the ring 4 and 5 carbons. The larger the substituents, the less nephrotoxic the substances appeared to be. In addition to being nephrotoxic, some of the thiazoles studied were also hepatotoxic. In the ring C4- and C5-substituted derivatives, ring substitution at the 2 position carbon (the one between the S and the N atom) markedly reduced the nephrotoxicity, but the hepatotoxicity was maintained, except when the substituted was a hydroxyl group (e.g. 2-hydroxy-4-methylthiazole). It was also demonstrated that thioformamide was both nephrotoxic and hepatotoxic, but only in GSH-depleted animals. Thioamides from ring C2 substituted thiazoles appeared to be only hepatotoxic, both after GSH-depletion and in normal animals, although in the normal animals the hepatotoxicity was less pronounced (Mizutani et al., 1993). Thus, the toxicity appears to be associated with GSH-depletion, which is a high-dose phenomenon.

In additional studies, again with mice, administration of another non-flavouring thiazole derivative with known hepatotoxic and nephrotoxic properties, 4-*t*-butyl-2-methylthiazole, at a dose of 312 mg/kg bw by gavage, resulted in limited ring C-oxidation. Only 0.25 % of the administered dose was recovered as a ring fragmentation product (3,3-dimethyl-2-oxobutanal; an alpha-dicarbonyl metabolite) in the urine within 24 hours. In addition, a thioamide metabolite (thioacetamide) was found; but this was not quantified. Other metabolites were not studied. Based on the structure of the dicarbonyl fragment, it was postulated that the 4,5-double bond of the thiazole ring undergoes epoxidation followed by hydrolysis to yield 4,5-diol. The diol then undergoes hydrolytic cleavage to yield the corresponding carbonyl derivatives and thioacetamide. Similar, thiazole ring opening has also been observed with thiabendazole (= 2-(thiazol-4-yl)benzimidazole) and with 2-(*p*-methoxyphenyl)-4-methylthiazole (Mizutani et al., 1994).

Additional information for candidate substances in Subgroup A-II

In an extensive review on the metabolism of heterocyclic substances, Rance (1989) indicated that several alternative metabolic pathways are available to thiazoles. One involves oxidation of *S* to form the corresponding sulphoxide and sulphone. Electrophilic attack at the ring N (resulting in quaternisation, e.g. N-methylation) is stated to be even more preferential than S-oxidation. The C2 carbon atom is electron deficient and substituents at this position are reactive and subject to nucleophilic displacement, in particular if the ring N is quaternised. Another pathway involves electrophilic attack at ring carbons, preferably C5. The C5 hydroxythiazoles exist as two tautomeric forms. C5-hydroxylation, however, is quantitatively unimportant because thiazoles do not readily undergo electrophilic substitution reactions (Rance, 1989).

Alkyl- and acyl-substituted thiazole derivatives can be metabolised *via* side-chain oxidation and ring *S*- and *N*-oxidation (Dalvie et al., 2002). The major metabolites are readily excreted in the urine either free or as glutathione conjugates. In addition, it may be expected that the presence of alkyl- and acyl-substituents at the thiazole ring increases the number of metabolic options. It has also been demonstrated that to a very small extent ring *C*-oxidation may be followed by ring cleavage to yield alpha-diketone and thioamide fragments (see Figure III.4). The acyl-substituted thiazoles [FL-no: 15.038, 15.039, 15.085 and 15.116] may also be expected to undergo keto-reduction, possibly followed by conjugation, similar to acetyl derivatives of furane (EFSA, 2011h) and pyrazine (EFSA, 2010h). However, there is very limited information to evaluate the extent of metabolism of ring substituent groups, and it is also very difficult, based on the information available, to assess how the presence of ring substituents and their positions at the ring might influence the metabolism of the ring itself.

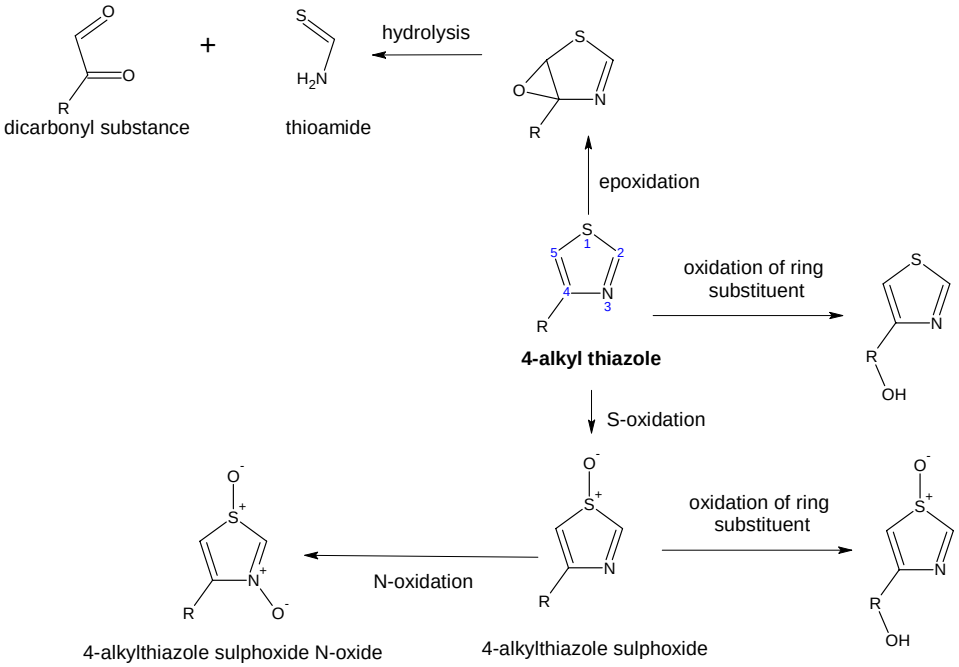


Figure III.4. *Metabolism of 4-alkylthiazols*

Subgroup A-III: Benzothiazoles

2-Methyl-4,5-benzothiazole [FL-no: 15.088]

No studies are available on the candidate substance in this group. However, there are some data on another (more complex) benzothiazole derivative and on benzothiazole [FL-no: 15.016]. The data are presented below:

Benzothiazole is primarily metabolised in guinea pigs by thiazole ring cleavage. A 30 mg/kg bw dose administered by intraperitoneal injection is metabolised to free or conjugated forms of *o*-aminophenyl methyl sulphide, *o*-aminophenyl methyl sulphoxide, and *o*-aminophenyl methyl sulphone. Small amounts of the *N*-hydroxy derivatives of the sulphoxide and sulphone were also detected (Wilson et al., 1991).

If substitution is present at the 2-position of the thiazole ring, depending on the nature of the substituent, hydroxylation of the benzene ring may be observed. In dogs, the calcium channel blocker fostedil (i.e. (4-(2-benzothiazolyl)phenyl) methylphosphonic acid diethyl ester), a 2-substituted benzothiazole, has been claimed to undergo benzene ring hydroxylation mainly at C5, C6 and C7, but experimental details were not available (Thomas, 1984). It is not clear if benzene ring hydroxylation would also apply to the one candidate substance in this group.

III.3.2. Non-aromatic candidate substances

Subgroup B-I: Dihydrothiophenes

4-Hydroxy-2,5-dimethylthiophen-3(2H)-one [FL-no: 15.077]

No data were available for the non-aromatic dihydrothiophene candidate substance [FL-no: 15.077].

Rance (1984) has drafted some general rules for the prediction of metabolism of sulphur-heterocycles. One of these rules is that with increasing level of ring saturation, the possibilities for S-oxygenation leading to sulphoxides and sulphones, will increase due to the lower effect of aromatic stabilisation, which might be relevant for the prediction of the metabolism of this candidate flavouring substance (Rance, 1989).

Subgroups B-II, B-III, B-IV, B-V and B-VI: Thiazoline, Thiazolidine, Dithiazine, Dihydrothiazine and Thiadiazine derivatives

Candidate substances [FL-no: 15.060, 15.086 and 15.119] (thiazolines from subgroup B-II),

Candidate substances [FL-no: 15.090 and 15.099] (thiazolidines from subgroup B-III),

Candidate substances [FL-no: 15.042, 15.054, 15.055, 15.057, 15.079 and 15.135] (dithiazines from subgroup B-IV),

Candidate substances [FL-no: 15.114 and 15.133] (dihydrothiazines from subgroup B-V)

Candidate substance [FL-no: 15.129] (a thiadiazine from subgroup B-VI)

No specific information was available to evaluate the metabolism of any of the candidate substances mentioned in these five subgroups (B-II, B-III, B-IV, B-V and B-VI).

It may be speculated that the substances in these chemical groups which contain a partially or completely reduced thiazole, a dithiazine or a reduced thiazine ring, are metabolised primarily by oxidation of the ring S or *via* N-oxidation, similar to the aromatic thiazole compounds. In addition, metabolism of the ring substituents is likely to occur.

III.4. Summary and Conclusions

The candidate substances in this FGE are structurally related to 29 flavouring substances evaluated by the JECFA in "Safety Evaluations of Groups of Related Flavouring Agents: Sulfur-Containing Heterocyclic Compounds". The candidate and 29 supporting substances in this group were subdivided into 11 subgroups based on the nature of the ring (aromatic (clustered in subgroups A-I to A-III) *vs.* non-aromatic (clustered in subgroups B-I to B-VI)), type and number of ring heteroatoms (sulphur or sulphur with nitrogen), and the degree of saturation in the non-aromatic rings.

For the evaluation of the metabolism of the candidate substances in this group, only very limited data were available. These were confined to a few references on thiophene, and some thiophene-, thiazole- and benzothiazole-derivatives (i.e. only directly relevant for the evaluation of subgroups-A, the aromatic candidate substances). Other information was found in several review papers. Virtually no data were found on the possible metabolism of the non-aromatic ring structures in the B-subgroups.

III.4.1. A-subgroups (substances with aromatic ring structures)

Very few data are available on absorption, distribution and excretion. Some of the studies are related to oral exposure and some others to parenteral dosing, and the relevance of the latter may be limited. In mice, a thiazolobenzimidazole derivative was rapidly absorbed after oral dosing, *S*-oxidised, and eliminated from plasma. The candidate substance thiophene has been shown to be absorbed rapidly from the gastro-intestinal tract and eliminated *via* urine as metabolites, or *via* the lungs. Also the thiazole derivative fostedil is absorbed from the gastro-intestinal tract (42 - 71 % of the dose), metabolised and eliminated with a plasma

half-life of about 6 - 9 hours in dogs. Hence, from the data on absorption and elimination that are available for the aromatic candidate substances in this FGE, it may be speculated that most of them will be fairly well absorbed and eliminated after biotransformation. Some volatile substances may also be eliminated via the lungs. For the non-aromatic candidate substances no information is available.

From the available metabolism data, it may be anticipated that the sulphur- and nitrogen containing heterocyclic and heteroaromatic derivatives participate in metabolic pathways, principally involving side-chain *C*-oxidation, oxidation of the ring *S* and ring *N* to yield sulphoxide or sulphones and *N*-oxides, respectively. Sulphoxide metabolites may conjugate with glutathione or may undergo dimerisation via a Diels-Adler reaction. For substituted thiophenes also epoxidation of the double bonds has been reported, which may also be followed by glutathione conjugation and mercapturic acid formation. As far as studied, ring *C*-oxidation may to a very minor extent be accompanied by heterocyclic ring cleavage, which for thiazoles could result in the formation of reactive thioamide intermediates. In addition to this cleavage of the heterocyclic ring, oxidation of the fused benzene ring has been reported for some structural analogues of the one candidate substance 2-methyl-4,5-benzothiazole [FL-no: 15.088] in subgroup A-III (benzothiazoles).

Additional alkyl and acyl substituents in the thiazole ring may increase the alternative metabolic pathways available to thiazole derivatives as was demonstrated for chlormethiazole. Alkyl- and acyl-substituted thiazole derivatives are primarily metabolised *via* side-chain oxidation and ring *S*- and *N*-oxidation. The major metabolites are readily excreted in the urine either free or as glutathione conjugates. Metabolites of the side-chain oxidation pathways may be expected to be conjugated e.g. to glucuronic acid. To a very small extent ring *C*-oxidation may be followed by ring cleavage to yield alpha-diketone and thioamide fragments. The acyl-substituted thiazoles [FL-no: 15.038, 15.039, 15.085 and 15.116] may also be expected to undergo keto-reduction, possibly followed by conjugation, similar to acyl derivatives of furanes (EFSA, 2011h) and pyrazines (EFSA, 2010h).

Thiophene (subgroup A-Ia) and the ring-substituted thiophenes (subgroups A-Ib and A-Ic) undergo *S*-oxidation and glutathione conjugation. Also from some studies with thiazole derivatives, it can be seen that their metabolites may react spontaneously with glutathione, and it is likely that they have also reactivity towards protein thiols, which may result in toxicity. E.g. for the thiazole-ring cleavage products (thioamide-intermediates) a relationship with nephrotoxicity and hepatotoxicity has been established, especially after GSH depletion, even though this is only a very minor metabolite.

Limited information was submitted that indicates that side chain oxidation of the alkyl- or acyl-substituted thiophenes (subgroups A-Ib and A-Ic) or thiazoles (subgroup A-II) may also occur. Such reactions may be anticipated (e.g. omega or omega-1 oxidations). Similar reactions have been discussed for alkylated pyrazines (EFSA, 2010h). In addition the two candidate substances with aldehyde groups [FL-no: 15.107 and 15.074] may be expected to be oxidised to the corresponding carboxylic acids. Where applicable, conjugation with amino acids (e.g. glycine) or glucuronic acid may also be expected to occur (EFSA, 2005e; EFSA, 2010h; EFSA, 2011h).

Based on the MSDI exposure estimates (see Table 6.1 of the main text) it seems reasonable to assume that saturation of the metabolic pathways for the candidate substances in this FGE is unlikely, given these low levels of exposure to the candidate substances from their use as flavouring substances.

Overall there is insufficient quantitative information to evaluate the extent of metabolism of ring substituent groups, and it is also very difficult, based on the data available, to assess how the presence of ring substituents and their positions at the ring influences metabolism of the rings itself, for any of the subgroups within this FGE. With respect to sulphydryl reactivity in thiophenes (subgroup A-Ic), this property was a reason to consider such substances, e.g. FGE.13, as not being metabolised through pathways leading to innocuous metabolites. In concordance with this and based on the information presented above, it is concluded that for all candidate flavouring substances in A-subgroups of the present FGE, it cannot be anticipated that they are metabolised to innocuous metabolites.

III.4.2. B-subgroups (substances with non-aromatic ringstructures):

No specific information was available on the metabolism of the dihydrothiophene-, thiazoline-, thiazolidine-, dithiazine-, dihydrothiazine- or thiadiazine-derivatives or of related substances for any of the (non-aromatic) substances in the B-subgroups. It may be speculated that the substances in these chemical groups are metabolised primarily by oxidation of the ring S which indeed was reported for the thiazolidine derivative already mentioned, or, if applicable, via N-oxidation, similar to the aromatic thiazole and thiophene compounds. In addition, metabolism of the ring substituents is likely to occur. Due to the lack of metabolism data on the substances in the B-subgroups, it cannot be concluded that the candidate flavouring substances in the respective B-subgroups will be metabolised to innocuous products.

ANNEX IV: TOXICITY

Oral acute toxicity data are available for two candidate substances of the present flavouring group evaluation and for 17 supporting substances evaluated by the JECFA at the 59th meeting. The supporting substances are listed in brackets.

Table IV.1: ACUTE TOXICITY

Chemical Name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference
Thiophene [15.106]	Mouse	M	Oral	1902	(O'Donoghue, 1979)
	Mouse	F	Oral	> 500	(Eli Lilly and Company, 1992)
	Rat	M	Oral	1131	(O'Donoghue, 1979)
	Rat	M, F	Gavage	3120	(Younger, 1964)
Thiophene-2-carbaldehyde [15.107]	Mouse	NR	Oral	565	(Sharp, 1979)
	Rat	NR	Oral	915 (950)	(Sharp, 1979)
(2-Thienyl disulfide [15.008])	Mouse	M, F	Gavage	400	(Moran et al., 1980)
(2-Methyl-5-methoxythiazole [15.002])	Rat	NR	Oral	1250	(Posternak et al., 1975)
(2,4-Dimethyl-5-vinylthiazole [15.005])	Mouse	M, F	Oral	400 – 800	(Moran et al., 1980)
(5-Acetyl-2,4-dimethylthiazole [15.011])	Mouse	M, F	Gavage	975	(Moran et al., 1980)
	Mouse	M, F	Gavage	M: 610, F: 620	(Shellenberger, 1971d)
(5-(2-Hydroxyethyl)-4-methylthiazole [15.014])	Mouse	M, F	Oral	4800	(Calvary and Nelson, 1944)
(4-Methyl-5-vinylthiazole [15.018])	Mouse	M, F	Gavage	400 – 800	(Oser, 1970c)
(2-Propionylthiazole [15.027])	Mouse	M, F	Gavage	2113	(Moran et al., 1980)
(4-Methylthiazole [15.035])	Mouse	F	Gavage	0.36 ml/kg (360 mg/kg, based on an assumed specific gravity of 1.0 g/ml)	(O'Neal et al., 1978)
	Rat	M, F	Gavage	930	(Mondino, 1981b)
(4,5-Dimethylthiazole [15.017])	Rat	M, F	Gavage	964.9	(Piccirillo et al., 1982a)
(2-Ethoxythiazole [15.021])	Rat	M, F	Gavage	910.9	(Piccirillo et al., 1982b)
(2-Isopropyl-4-methylthiazole [15.026])	Rat	M, F	Gavage	0.83 ml/kg (832 mg/kg based on a specific gravity of 1.003 g/ml)	(Griffiths and Babish, 1977b)
(2-Ethyl 4-methylthiazole [15.033])	Rat	M, F	Oral	540	(Moreno et al., 1981b)
(Thiamine hydrochloride [16.027])	Rat	M	Gavage	3710	(Sprince et al., 1974)
(Benzothiazole [15.016])	Mouse	M, F	Gavage	900	(Moran et al., 1980)
	Rat	M, F	Oral	380	(Birch, 1976a)
	Rat	M, F	Gavage	492	(Younger, 1964)
	Rat	M, F	Gavage	479	(Reddy and Mayhew, 1992)
	Rat	M, F	Oral	257 (M), 177 (F)	(Loeser, 1982a; Loeser, 1982b)
(2-(2-Butyl)-4,5-dimethyl-3-thiazoline [15.029])	Mouse	M, F	Gavage	2827	(Moran et al., 1980)
(4,5-Dimethyl-2-ethyl-3-thiazoline [15.030])	Mouse	M, F	Gavage	1265	(Moran et al., 1980)
(4,5-Dimethyl-2-isobutyl-3-thiazoline [15.032])	Mouse	M, F	Gavage	3067	(Moran et al., 1980)

Subacute / Subchronic toxicity data are available for three candidate substance of the present flavouring group evaluation and for 11 supporting substances evaluated by the JECFA at the 59th meeting. No chronic or carcinogenicity studies were available. The supporting substances are listed in brackets.

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
Thiophene [15.106]	Rat; M 5	Gavage	0, 50, 100, 500, 1000 mg/kg bw/day	Up to 19 days	100	(O'Donoghue, 1979)	Inadequately reported study of short duration.
	Rat; M/F 13	Gavage	0, 25, 100, 400 mg/kg bw/day	42	25	(Nagao, 2006)	Only a translated summary report available.
2-Pentylthiophene [15.096]	Rat; M/F 5	Gavage	0, 15, 150, 500 mg/kg bw/day	28	15	(Dhinsa et al., 2006)	
	Rat; M/F 5	Gavage	0, 3 mg/kg bw/day	28	3	(Marr and Watson, 2007)	
Thiophene-2-carbaldehyde [15.107]	Rat; sex not reported 5	Diet	0, 0.1, and 1.0 % in diet equal to 0, 95 and 840 mg/kg bw/day	11 days	95	(Sharp, 1979)	Reduced food consumption and reduced absolute kidney weight. Inadequately reported study of short duration.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Rat; M, F 5/sex/group	Diet	10 mg/kg bw/day	14 days	10	(Gill and Van Miller, 1987b)	One dose level, GLP study, comprehensively reported, but no haematology or clinical chemistry carried out, only a limited number of organs examinaed at autopsy and only liver and kidney examined histopathologically. Short duration, limited study design.
(3-Acetyl-2,5-dimethylthiophene [15.024])	Rat; M, F 5/sex/group	Diet	10 mg/kg bw/day	14 days	10	(Gill and Van Miller, 1987b)	One dose level, GLP study, described as a dietary Minimum Toxicity Screen (MTS), comprehensively reported, but no haelatology or clinical chemistry carried out, only a limited number of organs examinaed at autopsy and only liver and kidney examined histopathologically. Minor body weight changes in both sexes and organ weight changes in males only. Short duration, limited study design.
(2-Thienyl disulfide [15.008])	Rat; M, F 15/sex	Diet	0.29 mg/kg bw/ day	90 days	0.29	(Morgareidge and Oser, 1970g)	Study carried out at a one low dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Parameters assessed were reasonably comprehensive. Study considered valid.
(5-Acetyl-2,4-dimethylthiazole [15.011])	Rat; M, F 23/sex	Diet	approx. 25 mg/kg bw/day.	90 days	M: 25.2 F:24.4	(Shellenberger, 1971d)	Old study carried out at a one dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Parameters assessed were reasonably comprehensive. Study considered valid.
(2-Acetylthiazole [15.020])	Rat; M 5	Diet	5000 and 10,000 mg/kg in diet equivalent to 250 and 500 mg/kg bw/day	28 days	< 250	(Wheldon et al., 1970)	Preliminary dose-range finding study for the 90-day study described below, very limited experimental details provided.
	Rat; M 10	Diet	0. 100, 1000, 10,000 mg/kg in diet equivalent	90 days	50	(Wheldon et al., 1970)	Study considered to be of limited validity, since no clinical chemistry was undertaken, limited number of organs weighed, number of organs taken for histopathology was limited, and histopathological review

Table IV.2: Subacute / Subchronic / Chronic / Carcinogenicity Studies

Chemical Name [FL-no]	Species; Sex No./Group	Route	Dose levels	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
			to 0.5, 50, 500 mg/kg bw/day				restricted to 5 animals/sex/group.
(2-Methyl-5-methoxythiazole [15.002])	Rat; M, F 10-16/sex	Diet	approx. 9 mg/kg bw/day	90 days	M: 8.83 F: 8.63	(Posternak et al., 1975)	Toxicity data reported as part of a summary publication on a large number of flavouring substances. Body weight, food utilisation, haematological and histopathology examination undertaken, clinical chemistry restricted to blood urea nitrogen, organ weight changes restricted to liver and kidney. Due to reporting limitations data quality could not be assessed.
(2,4-Dimethyl-5-vinylthiazole [15.005])	Rat; M, F 10-16/sex	Diet	approx. 1 mg/kg bw/day	90 days	M: 0.92 F: 1.0	(Posternak et al., 1969)	Toxicity data reported as part of a summary publication on a large number of flavouring substances. Body weight, food utilisation, haematological and histopathology examination undertaken, clinical chemistry restricted to blood urea nitrogen, organ weight changes restricted to liver and kidney. Due to reporting limitations data quality could not be assessed.
(Benzothiazole [15.016])	Rat; M, F 15/sex	Oral	5.1 mg/kg bw/ day	90 days	5.1	(Morgareidge, 1971c)	Body weight, food utilisation, haematology and limited clinical chemistry parameters were evaluated, and a range of tissues was examined microscopically. No treatment-related effects were seen. Only the histopathological report was available for the current evaluation. The study is considered valid.
2-(2-Butyl)-4,5-dimethyl-3-thiazoline [15.029])	Rat; M, F 15/sex	Oral	1.21(M) /1.26 (F) mg/kg bw/ day	90 days	M: 1.21 F: 1.26	(Babish and Re, 1978)	Old study carried out at a one dose level. Standard toxicological parameters were monitored (body weight, food consumption, haematology, urinalysis, histopathology). Study considered valid.
(2- Isobutyl-4,6-dimethyldihydro-1,3,5-dithiazine and 4-isobutyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	Rat, M, F 5/sex	Oral	10 mg/kg bw/day	14 days	M: 11.5 F: 11.1	(Rush, 1989a)	One dose level study of short duration, no haematological or clinical chemistry parametes were examined and histopathological examinations were restricted to liver and kidney.
(2- Isopropyl-4, 6-dimethyldihydro-1,3,5-dithiazine and 4-isopropyl-2,6-dimethyldihydro-1,3,5-dithiazine (mixture))	Rat, M, F 5/sex	Oral	10 mg/kg bw/day	14 days	M: 11.8 F: 11.1	(Rush, 1989b)	One dose level study of short duration, no haematological or clinical chemistry parametes were examined and histopathological examinations were restricted to liver and kidney.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Developmental and reproductive toxicity data are available for one candidate substance of the present Flavouring Group Evaluation and for none of the supporting substances evaluated by the JECFA at the 59th meeting.

TABLE IV.3: DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Chemical Name [FL-no]	Species/ Sex No./ group	Route	Dose Levels	Duration	NOAEL (mg/kg bw/day), Including information of possible maternel toxicity	Reference	Comments
Thiopene [15.106]	Rats/M, F 13	Gavage	0, 25, 100, 400 mg/kg bw/day	42 days	M: 400 mg/kg bw F: 25 mg/kg bw	(Nagao, 2006)	Only a translated summary report available.

In vitro mutagenicity/genotoxicity data are available for 12 candidate substances of the present flavouring group evaluation from chemical group 29 or 30 and for four supporting substances evaluated by the JECFA at the 59th meeting and four related supporting substances. Supporting substances are listed in brackets.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
Subgroup A-Ia						
Thiophene [15.106]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA1535; TA1537	3 µmol/plate (all strains) (252 µg/plate)	Negative (±S9)	(Florin et al., 1980)	Published non-GLP study. Qualitative screening in a spot-test with three strains, quantitative study (4 doses, 0.03, 0.3, 3, 30 µmol/plate) with TA 100 only. Limited report of experimental details and results. Insufficient quality, study not considered adequate for the evaluation of mutagenic activity.
	Ames assay (preincubation method)	*S. typhimurium* TA97;TA98; TA100; TA1535; TA1537	Up to 10,000 µg/plate	Negative (±S9)[1]	(Zeiger et al., 1987)	Non-GLP study roughly in accordance with OECD guideline 471. The study is considered valid.
	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01-1.2 mmol/plate (100,968 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (8414 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
	Ames assay	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA	0, 78.1, 156, 313, 625, 1250 µg/plate 0, 78.1, 156, 313, 625, 1250,2500, 5000 µg/plate	Negative (±S9) Negative (±S9)	(Shibuya, 2006)	Valid study according to OECD Test Guidelines and Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
	Chromosomal Abberation	Chinese hamster lung cells	0, 210, 420, 840 µg/ml	Negative (±S9)	(Tanaka, 2006)	Valid study according to Guidelines for screening mutagenicity testing of chemicals (Japan), provided as a translation of the original report in Japanese.
Subgroup A-Ib						
2-Methylthiophene [15.091]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.00001- 1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
3-Methylthiophene [15.092]	Ames assay (preincubation method)	*S. typhimurium* TA98; TA100; TA102	0.01-1.0 mmol/plate (98,170 µg/plate)	Negative (±S9)	(Aeschbacher et al., 1989)	Greatest effects are quantified by "mutation factor," no numbers are given for negative results. Limited quality (only 3 strains used), but otherwise acceptable study.
	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9817 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
2,5-Dimethylthiophene [15.064]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11,219 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
2-Acetylthiophene [15.040]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
2-Acetyl-3-Methylthiophene [15.037]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
Thiophene-2-carbaldehyde [15.107]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
5-Ethylthiophene-2-carbaldehyde [15.074]	SOS Chromotest	*E. coli*	NR	Negative with rat S9, positive without rat S9	(Mosier et al., 2003)	Study endpoint inappropriate for the estimation of genotoxic potential.
(5-Methyl-2-thiophenecarbaldehyde [15.004])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (12,618 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
Subgroup A-II						
2,4-Dimethylthiazole [15.062]	Ames assay (plate incorporation method)	*S. typhimurium* TA100	9.3 and 94 mmol/l top agar (10,639 µg/ml)	Negative (-S9)	(Voogd et al., 1983)	Insufficient quality (one test strain as well as without metabolic activation only).
(4,5-Dimethylthiazole [15.017])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (11,318 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
(4-Methylthiazole [15.035])	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100	Up to 100 µmol/plate (9916 µg/plate)	Negative (±S9)	(Lee et al., 1994a)	Only two strains used but otherwise acceptable study.
Subgroup A-III						
2-Methyl-4,5-benzothiazole [15.088]	Ames assay (plate incorporation method)	*S. typhimurium* TA98; TA100; TA102; TA1535; TA1537	100-10,000 µg/plate	Negative (±S9)[1]	(Longfellow, 1998a)	Summary report of NCI-short-term test program, results not given in detail.
(Benzothiazole [15.016])	Ames assay	*S. typhimurium* TA98; TA100; TA1535; TA1537	Up to 5000 µg/plate	Negative (±S9)	(Bayer, 1991)	Summary in IUCLID data set only. According to this summary, the assay was in compliance with GLP; accordance with OECD guideline 471 not stated.
	Mouse lymphoma assay	Mouse L5178Y tk+/- cells	10-250 µg/ml	Negative (±S9)	(Longfellow, 1997)	Summary report of NCI-short-term test program, results not given in detail.
Subgroup B-III						
2-Propylthiazolidine [15.099]	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA98 and TA100.(±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
2-Methylthiazolidine [15.090]	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100; (±S9) 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Ethylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (±S9) and TA98 (-S9); 10 µg/ml: positive in	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

Table IV.4: GENOTOXICITY (*in vitro*)

Chemical Name	Test System	Test Object	Concentration	Result	Reference	Comments
				TA100 (±S9); 100 µg/ml: positive TA98 and TA100. (±S9)		
(2-Isopropylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 and 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Butylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA100 (+S9); 10 µg/ml: positive in TA100 (±S9); 100 µg/ml: positive in TA100 (±S9) and TA98 (-S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).
(2-Isobutylthiazolidine)	Ames assay	*S. typhimurium* TA98; TA100	1, 10, 100 µg/ml	1 µg/ml: positive in TA98 and TA100 (+S9); 10 µg/ml: positive in TA98 and TA100 (±S9); 100 µg/ml: positive in TA98 and TA100 (±S9)	(Mihara and Shibamoto, 1980)	The results were stated to be positive, however, the magnitude and a positive dose effect relationship could not be assessed (no numbers are given).

NR Not Reported.
1 With and without rat and hamster S9 metabolic activation.

TABLE IV.5: GENOTOXICITY (*IN VIVO*)

No *in vivo* mutagenicity/genotoxicity data are available neither for the candidate substance of the present flavouring group evaluation nor for the supporting substances evaluated by the JECFA at the 59th meeting.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
BW	Body Weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids Chemical Abstract Service
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
EC	European Commission
EFFA	European Flavour and Fragrance Association
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
GLP	Good Laboratory Practice
GSH	Glutathione
FLAVIS (FL)	Flavour Information System (database)
HPLC	High-performance liquid chromatography
ID	Identity
IOFI	International Organization of the Flavour Industry
IP	Intraparenteral
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD_{50}	Lethal Dose, 50 %; Median lethal dose
MS	Mass spectrometry
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
MTS	Minimum Toxicity Screen
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, reduced form
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level

NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
SC	Structural class
SCE	Sister Chromatid Exchange
SCF	Scientific Committee on Food
SMART	Somatic Mutation and Recombination Test
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA Synthesis
WHO	World Health Organisation